ACS Chemical Neuroscience

REVIEW

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Fragile X Syndrome: An Update on Developing Treatment Modalities

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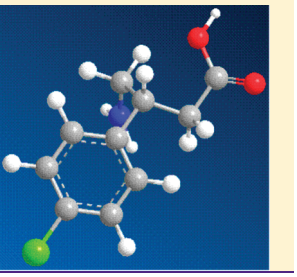
ABSTRACT: Intellectual disability (ID; mental retardation) is considered an immutable condition. Current medical practices are aimed at relieving symptoms and not at altering the underlying cognitive deficits. Scientific advancements from the past decade have led to the exciting possibility that ID may now be treatable. Moreover, pharmaceutical therapies targeting the most common form of inherited ID, Fragile X syndrome (FXS), may become the new benchmark for central nervous system (CNS) drug discovery: seeking cures for neurodevelopmental disorders.



KEYWORDS: Fragile X syndrome, fragile X mental retardation gene, fragile X mental retardation protein, metabotropic glutamate receptor 5, γ-aminobutyric acid

Translating our understanding of the core pathophysiology of Fragile X syndrome (FXS) into treatments for this patient population constitutes a paradigm shift for central nervous system (CNS) drug development. Defining the molecular pathways leading to neurodevelopmental disorders, in addition to identifying therapeutic targets, enables discovery of companion diagnostics and objective efficacy end points, molecular measures of disease susceptibility and treatment response. FXS, a single gene disorder that is a leading known cause of autism, may also be a model for autism research, providing a basis to ask whether there is a shared pathophysiology that is responsive to treatment.

FRAGILE X SYNDROME (FXS)

FXS was first described in pedigrees showing intellectual disability (ID) with X-linkage.[1] The name Fragile X syndrome stems from the cytogenetic assay optimized for the associated marker X chromosome that appeared as a characteristic constricted region or fragile site on the long arm of the X chromosome at position Xq27.3.[2] The single gene ultimately demonstrated to be responsible for FXS was discovered using positional cloning.[3] The FXS mutation is an expansion of a trinucleotide repeat in the 5′ untranslated region (UTR) of the fragile X mental retardation 1 gene (*FMR1*). Expansion occurs in the general population and is considered normal up to 40−50 CGG repeats; a premutation condition occurs between approximately 55 and 200 repeats. A diagnosis of FXS is confirmed with expansions greater than 200 CGG repeats. These CpG islands become methylated, which prevents *FMR1* transcription and results in the loss-of-function mutation.

Because *FMR1* is an X-linked gene, FXS is more prevalent in males (1:4000) than females (1:8000).[4] However, inheritance of FXS is complicated by the mechanism of the trinucleotide repeat expansion. Expansion is transmitted through the mother. Therefore, a carrier mother (one with a premutation) may have unaffected, carrier, or affected children. A carrier father will have only all carrier daughters and all unaffected sons. Once diagnosed, it is important to learn the inheritance pattern for treatment, prevention, and planning.

Neurological manifestations vary widely and include developmental delays, cognitive disabilities, autism, and seizures. Individuals can show hypersensitivity to tactile stimulation or other environmental stimuli. Repetitive behaviors such as hand flapping and perseverative speech are typical. Males often present with a characteristic physical appearance, which can include a long face with prominent ears and macroorchidism. Alterations in connective tissue are common and manifest as loose skin and joints. An altered gait may also be found. Females are generally less affected[5] due to mosaicism in X-chromosome inactivation, but they show a spectrum of impairments in intellectual ability from mild learning disabilities to ID.

FRAGILE X PREMUTATION

Premutation carriers, initially thought to be free of clinical symptoms, show distinct phenotypes.[6] In approximately 20% of carrier females, premature ovarian insufficiency (POI) occurs, a condition associated with early menopause, and in males a late-onset neurodegenerative condition called Fragile X tremor ataxia syndrome (FXTAS) occurs. FXTAS is characterized by intention tremor and ataxia in men over 50. The motor disorders can be accompanied by a progressive decline in cognitive function, aberrant behavior including anxiety and mood alterations and dementia.[7] Expansion to the premutation is more prevalent than occurrence of the full mutation, approximately 1:300 for females and 1:800 for males. The results of recent studies suggest an association of lifelong mood and anxiety disorders in premutation carriers.[8]

The neuropathology associated with FXTAS reveals nuclear inclusions in neurons and astrocytes. Although the contents of nuclear inclusions contain *FMR1* mRNA, ubiquitin, RNA-binding proteins, and nuclear proteins,[9,10] the role, if any, in disease progression remains unclear. These observations, along with the demonstration of increased *FMR1* mRNA levels in premutation carriers, led to the disease hypothesis that mRNA toxicity contributes to carrier phenotypes.[11,12] However, in murine models, the premutation not only resulted in increased *Fmr1* mRNA but decreased fragile X mental retardation protein

Received: March 2, 2011
Accepted: March 22, 2011
Published: March 22, 2011

(FMRP) and altered behavior, dendrite morphology, and protein synthesis.[13] The similar phenotypic alterations in the premutation mouse as compared to the *Fmr1* knockout mouse suggest that the decreased levels of FMRP, not increased *FMR1* mRNA, explain the clinical manifestations of premutation carriers. Although additional work is required to support this hypothesis, understanding the function of *FMR1*/FMRP in FXTAS may reveal common molecular pathways in other neurodegenerative diseases and lead to novel mechanism-based therapies.

FMRP STRUCTURE AND FUNCTION

Almost two decades of scientific investigation reveal FMRP to be a multifunctional protein regulating RNA translation in distinct subcellular compartments. Analysis of FMRP structural motifs led to studies focusing on FMRP function in nuclear binding and export of nascent RNAs, active transport of cargo mRNAs through the cytoplasm to dendritic spines, and local regulation of protein synthesis in response to neurotransmitter signals.[14−16]

The molecular mechanisms by which FMRP controls protein synthesis are complex, occur at multiple steps in the synthetic pathway, and are still not completely elucidated. FMRP contains three RNA-binding motifs that interact with specific RNA target elements. Two KH-type domains and a RGG domain interact with a sequence-specific element termed the FMRP kissing complex and the intramolecular G-quartet, respectively.[17,18] FMRP is part of RNA-protein granules or ribonuclear particles (mRNP) that associate with polyribosomes.[19] The FMRP-containing mRNPs shuttle target RNAs to dendritic spines. A spontaneously occurring point mutation in human FMRP, I304N, occurs in one of the KH2 domains and prevents FMRP from associating with actively translating polyribosomes. The results of studies examining the I304N mutation demonstrate the KH2 domain of FMRP represses protein translation through direct binding to the mRNA target.[20,21] FMRP-containing mRNPs actively translocate to dendritic spines via the microtubulin cytoskeleton.[22−25] Once positioned at dendritic spines, FMRP regulates protein synthesis through a mechanism of RNA interference. FMRP associates with dicer and the RNA-interfering complex (RISC). Dicer processes small interfering RNAs (siRNAs) and microRNAs (miRNAs), two noncoding RNAs. Target-mRNA-bound siRNAs and miRNAs incorporate into the RISC complex, where either the target mRNA is degraded or translation is inhibited.[26−28] Experimental evidence suggests that FMRP recruits these small RNAs to their mRNA targets.[29] Although several FMRP-mRNA targets have been identified, including *FMR1*-mRNA itself, the complete list is still under active investigation.

THE MGLUR THEORY OF FXS

A neuroanatomical alteration common to some forms of ID, including FXS, is abnormal dendritic spine morphology.[30] Characterization of an *Fmr1*-deficient mouse showed this same neuroanatomical phenotype was conserved across species and provided a tractable model to investigate the underlying neuropathology.[31] The dendritic spine is one point of contact for excitatory synapses, and synaptic connectivity is essential for experience-dependent learning and memory. Synaptic plasticity is bidirectional; connections are continuously strengthened and weakened. The group I metabotropic glutamate receptors, mGluR1 and mGluR5, modulate a form of synaptic weakening called mGluR-dependent long-term depression (LTD).[32,33] mGluR-LTD requires synaptic protein synthesis.[34,35] FMRP is a negative regulator of synaptic protein synthesis and is synthesized locally in response to group I mGluR activation.[36] The initial assumption was that FMRP was required for mGluR-LTD; however, analysis of synaptic plasticity in the *Fmr1*-deficient mouse showed that *Fmr1*-deficiency caused exaggerated LTD.[37] This finding led to the hypothesis that FMRP limits LTD by countering mGluR-dependent synaptic protein synthesis.[38] Based on this theory, reducing group I mGluR signaling either genetically or pharmacologically will reduce synaptic protein synthesis and in turn correct the fragile X mutant phenotype.

A genetic approach to test the mGluR theory of fragile X showed correction of all of the neurologic phenotypes measured. Stimulus-induced seizures, the excessive hippocampal protein synthesis, the exaggerated LTD, and the increased dendritic spine density were all reduced to normal levels when *Fmr1*-deficient mice were crossed with mice expressing a 50% reduction in mGluR5.[39] Reduction of mGluR5 also corrected the rate of inhibitory avoidance extinction, a measure of excessive memory extinction.

Extensive pharmacologic evidence from independent laboratories further supports the mGluR theory of fragile X. In addition to inhibition of induced seizures, the widely used mGluR5 negative allosteric modulator 2-methyl-6-(phenylethynyl)-pyridine (MPEP)[40] reduced prolonged epileptiform discharges in acute brain slices from *Fmr1*-deficent mice.[41] The excessive protein synthesis measured by metabolic labeling in *Fmr1*-deficient mice was also reduced with MPEP treatment, and alterations in the polysome and RNA granule (ribosomal clusters) fractions were corrected with MPEP administration, suggesting FMRP not only regulates mGluR5-induced protein translation but also affects the translational machinery.[42,43]

Collectively, these studies provide compelling evidence that altering mGluR5-dependent protein synthesis may directly affect the synaptic alterations underlying cognitive and behavioral phenotypes associated with this syndromic disorder.

ADDITIONAL FXS TARGETS AT THE SYNAPSE

Altered neurotransmission via the ionotropic glutamate receptors has recently been reported. In *Fmr1*-deficient mice, decreases in the amplitude of *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory postsynaptic currents were measured in the dentate gyrus of hippocampal slices[44] and NMDA receptor subunits were decreased in prefrontal cortex.[45] Reduced GluR1 and GluR2 α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunits were also measured in *Fmr1* cortex,[46,47] and excessive internalization of AMPA receptors is linked to synaptic weakening and altered dendrite morphology in *Fmr1*-deficient mice.[48]

In recent years, the importance of γ-aminobutyric acid (GABA) inhibitory transmission in FXS has emerged. In the *Fmr1*-deficient mouse, region-specific alterations in the GABAergic pathway exist. These include decreased synaptic and tonic GABAergic inhibition in the amygdala, the latter of which is reversed using the ionotropic $GABA_A$ receptor agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol[49] and decreased interneuron number in cortex but not hippocampus.[50] In contrast, increased GABAergic tone is observed in the striatum of *Fmr1*-deficient mice and likely results from an increase in the probability of GABA release.[51] The relative balance of excitation and inhibition may also be altered in FXS. In the fragile X fly model, dfmr-deficient *Drosophila* fed on a high glutamate diet die during

development and GABA-treated dfmr mutant flies show correction of several mutant phenotypes.[52] Studies examining induced seizure activity in mice suggest the opposing actions of mGluRs and $GABA_B$ receptors may provide a therapeutic path for FXS. Pacey and colleagues showed that, in wild type mice, CGP 46381, a $GABA_B$ antagonist, coadministered with CDPPB, an mGluR5 agonist, induces audiogenic seizures, while either compound alone had no effect. Of note, the $GABA_B$ agonist R-baclofen rescues the audiogenic seizure phenotype in *Fmr1*-deficient mice.[53] These results suggest $GABA_B$ receptor activation counters the effects of mGluR5 in FXS.

The opposing actions of GABA and glutamate have recently been investigated in a murine model proposed for autism research, the oxytocin receptor deficient mouse. Oxytocin is a neuropeptide involved in learning and memory and linked to social behavior. Oxytocin receptor deficient mice show deficits in social recognition, increased aggression and are susceptible to seizures.[54] Reduced GABAergic synapses were observed in cultured hippocampal neurons from oxytocin receptor-deficient mice, suggesting an imbalance in GABA/glutamate transmission underlies the behavioral deficits.[55]

The widely used antibiotic, minocycline, also recognized for its neuroprotective and anti-inflammatory effects on autoimmune diseases such as rheumatoid arthritis, has recently been applied to FXS. In a single study, minocycline showed a beneficial effect on dendritic spine morphology and improved behavioral performance in *Fmr1*-deficient mice.[56] The proposed mechanism of action is thought to be reduction of elevated matrix metalloproteinase-9 activity, which may play a role in the synaptic remodeling associated with plasticity.

Lithium, commonly used to treat bipolar disorder, has recently been tested as a treatment for FXS. Lithium inhibits phospholipase C and glycogen synthase kinase-3β (GSK-3β).[57] GSK-3β is widely expressed in brain, present at high levels in hippocampus, and its enzymatic activity is elevated in the FXS mouse brain.[58] FXS mice treated with lithium showed reversal of some behavioral phenotypes and partial correction of the dendritic spine phenotype.[59,60]

Additional targets implicated from studies of the *Fmr1*-deficient mouse include acetylcholine (ACh) transmission through the muscarinic acetylcholine receptors. Muscarinic-acetylcholine-receptor-stimulated LTD and protein synthesis were altered in *Fmr1*-deficient mice, suggesting cholinergic-dependent synaptic plasticity is signaled in parallel with the mGluR5 pathway.[61] Finally, two enzymes, the small GTPase Rac1 and associated p21-activated kinase (PAK), that regulate actin polymerization in dendritic spines and are thought to play a role in plasticity, have been identified as potential therapeutic targets for FXS. Dysregulation of Rac1 and the associated PAK were identified in the *Fmr1* mouse, and inhibition of PAK reverses some of the mutant phenotypes.[62,63]

MEDICINAL CHEMISTRY OF MGLUR5 NEGATIVE ALLOSTERIC MODULATORS

Based on the mGluR theory, there continues to be strong interest in mGluR5 antagonists as a potential therapeutic approach to FXS. Indeed, the focus of medicinal chemistry efforts that could contribute to new pharmacologic agents for the treatment for FXS have largely focused in this area. Several reviews[64−67] have appeared in the past few years describing efforts to design effective negative modulators of mGluR5 suitable for clinical development, including a comprehensive medicinal chemistry review in 2009 by Lindsley and Emmitte.[68] Those reviews also covered emerging thoughts around the therapeutic utility of mGluR5 antagonists as well. More recent medicinal chemistry activities in the mGluR5 arena will be described herein. It should first be noted that a great deal of the preclinical work surrounding mGluR5 antagonists has been carried out in the context of disease applications other than FXS, such as anxiety, pain, GERD, and others. It is generally considered that new mGluR5 antagonists could have many potential applications, including in FXS where a strong mechanistic rationale exists. Importantly, early clinical trials in FXS have shown some promise, including encouraging results from both an open label trial with fenobam (compound **1**, Figure 1)[69] as well as a randomized double-blind trial with the Novartis compound AFQ056.[70] With the exception of fenobam, specific chemical structures of the most advanced compounds for FXS have not been revealed in the literature. As noted in aforementioned reviews, much of the chemistry in the mGluR5 antagonist arena has focused on modification of the early prototype alkyne-containing compounds MPEP (compound **2**) and 3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP, compound **3**). More recent medicinal chemistry activity has for the most part diverged from 1,2-diarylalkyne analogues and has focused on novel chemical scaffolds, resulting from either rational drug design or new high throughput screening campaigns. In the former case, the structure−activity relationship (SAR) around known pharmacophores derived from studies on MPEP and MTEP continues to be employed, as demonstrated in the first three papers referenced below.

In an extension of previous work,[71,72] a group from the National Institute on Drug Abuse (NIDA) presented new aryl quinolines and aryl benzothiazoles,[73] wherein rigid nonalkyne analogues of MPEP and MTEP were optimized. In this work, it was demonstrated that the SAR of MPEP could effectively be applied to the new scaffolds, yielding potent noncompetitive antagonists with EC50s in the range of 60−100 nM in vitro (e.g., compound **4**). In vivo work on these compounds has yet to be reported.

In yet another example of furthering the understanding around known pharmacophores useful for antagonizing the mGlu5 receptor, the Vanderbilt group investigated the SAR of a series of compounds anchored by 3-cyano-5-fluoro-benzamides (structure **5**), a substructure known to impart good potency in other series of mGluR5 antagonists.[74] Two potent compounds were characterized, a methylthiazole analogue (compound **6**) and a chlorophenyl analogue (compound **7**), and shown to have good pharmacokinetics (PK) characteristics upon IP dosing in rats.

A more traditional rational drug design approach was undertaken by Burdi et al.[75] starting with known pharmacophores from early mGluR5 antagonist programs at Novartis and Merck as the basis to design new chemotypes, including a number of analogues around an oxazolopiperidine structure. Further elaboration of this scaffold ultimately led to a very potent oxazolo-axepine (compound **8**). Note that compound **8** also contains the aforementioned 3-cyano-5-fluoro aniline substructure. This compound, despite showing good brain penetration and high receptor occupancy, was not sufficiently robust from a PK standpoint for further development.

A good example of new chemical scaffolds arising from high-throughput screening (HTS) comes from the Vanderbilt group, where several non-MTEP chemotypes have been reported. A novel series of anilinoquinazolines (e.g., compound **9**) were reported[76] to demonstrate good potency against mGluR5, and though they do not resemble MTEP, they, like most mGluR5

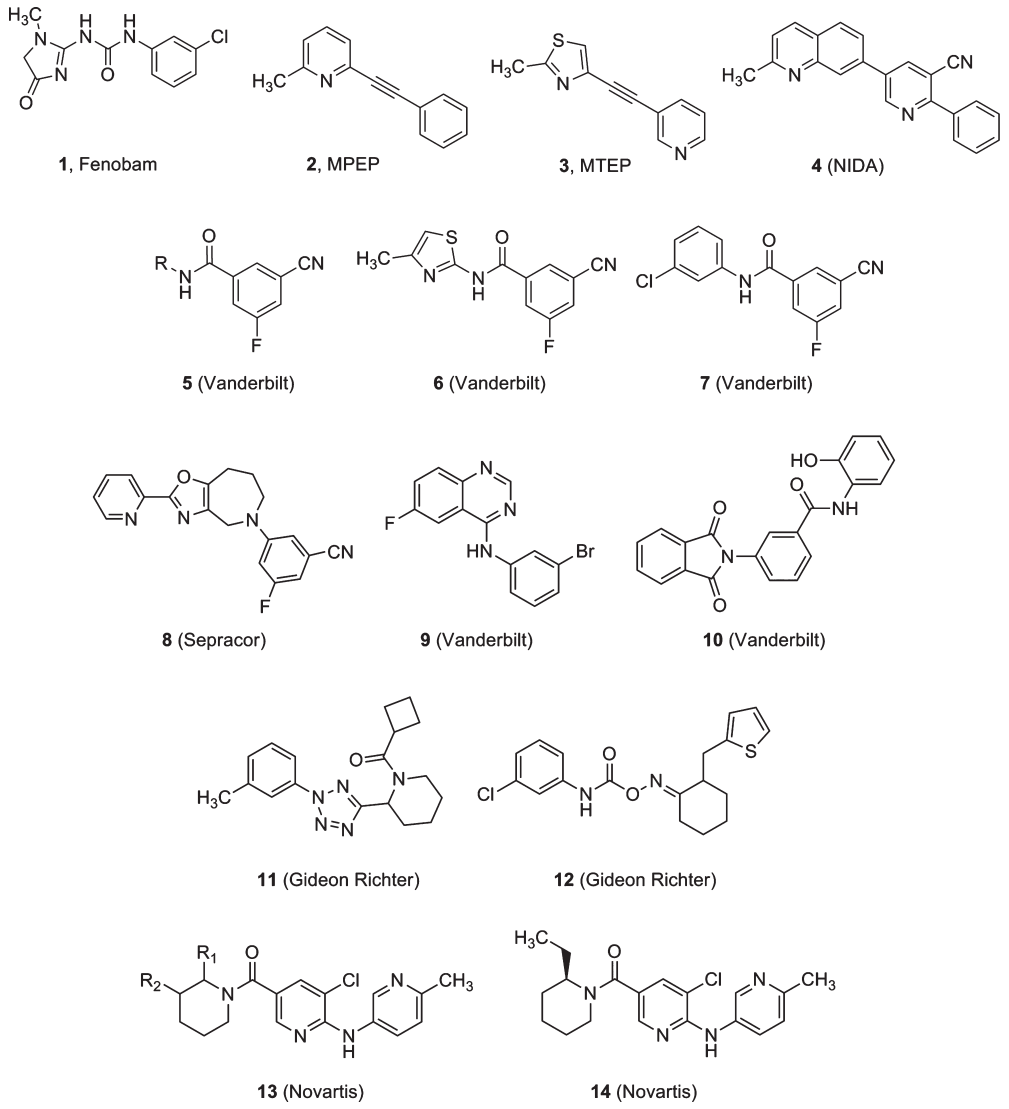


Figure 1. Structures of prototypical mGluR5 NAMs **1**−**3**, and novel chemotypes **4**−**14** indicating the company/university that developed them.

antagonists of any structural chemotype, still bind at the MTEP site as revealed by inhibition of binding of a radioligand at that site. A second report from that group[77] detailed potent benzamide analogues (e.g., compound **10**), also reported as being derived from an HTS run. Again, these novel chemotypes appear to bind at the MTEP site. Both of these reports focus on early hit elaboration and on understanding the pharmacology of the new chemotypes. Complexities are clearly evident when attempting to correlate structure to mode-of-binding activities.

Two reports also came out of the laboratories of Gideon Richter describing hit-to-lead efforts around new HTS hits. The first[78] describes novel tetrazole compounds (compound **11**), and the second[79] describes potent carbamoyloximes (compound **12**), resulting in low nanomolar mGluR5 antagonists, with some early CYP optimization. These compounds are at the early stages of elaboration.

An additional description of a novel scaffold arising from HTS was reported by the Novartis group.[80] A series of piperidyl amides, characterized by structure **13**, were expanded to ultimately yield a potent compound (compound **14**), wherein R1 is substituted with an ethyl group with the R configuration (the R analogue being about 10× more potent than the S analogue). In vitro radioligand displacement assays showed that compound **14** bound to the previously characterized allosteric binding on mGluR5. Compound **14** displayed good pharmacokinetic parameters in the rat, showed good efficacy in three rat anxiety models, and had a good PK/PD correlation as well. The authors state that this compound was considered for further development.

It is yet to be seen whether compounds resulting from rational drug design efforts or HTS-derived novel scaffolds can be elaborated into advanced lead compounds with requisite properties for advancement into preclinical and ultimately clinical trials. However, it is an exciting time for the discovery and development of mGluR5 antagonists for FXS, as multiple compounds are advancing in clinical trials.

THERAPEUTIC INTERVENTIONS

Arbaclofen. The biological action of the racemic drug baclofen, used clinically as an antispastic agent for over 30 years, is known to reside with the active *R*-enantiomer or arbaclofen.[81−86] It is exerted through the metabotropic $GABA_B$ receptor for the amino acid, GABA, which is recognized as the main inhibitory

neurotransmitter in the CNS.[83,87−89] Of relevance to the treatment of FXS, baclofen inhibits the neuronal presynaptic release of glutamate which in turn acts to block downstream signaling from mGluR5.[90−92] In addition, specific deficiencies in GABA neurotransmission have been found in FXS[49] that arbaclofen treatment could also possibly correct. Seaside Therapeutics currently has clinical trials ongoing with arbaclofen or STX209 in patients with FXS and autism spectrum disorders (http://www.seasidetherapeutics.com/).

Modulating Glutamate in FXS. A number of potential therapeutic interventions targeting FXS have been investigated in recent years, and many focused on modulation of glutamate signaling in the brain.

The possible contribution to FXS pathophysiology of dysregulated glutamate receptor activity through the ionotropic pathway involving the NMDA receptor was studied in an open-label clinical trial using the NMDA receptor antagonist, memantine.[93,94] From a total of 6 patients studied, 4 showed global clinical benefit as measured on the Clinical Global Impression-Improvement subscale (CGI-I) over an approximate 35 week treatment period while 2 of the patients developed treatment-limiting irritability.[95] Spurred by these findings, The University of California, Davis in collaboration with Forest Laboratories and the National Institute on Aging are recruiting a randomized, double-blind, placebo-controlled study in 180 Fragile X premutation carriers (CGG repeat 55−200) that have neurological symptoms (http://clinicaltrials.gov/ct2/show/NCT00584948?term=Fragile+X+syndrome&rank=26). Neurocognitive end points are being utilized as outcome measures.

AMPA receptor down regulation has been implicated as another contributory mechanism to glutamate receptor imbalance in FXS.[96] Cortex Pharmaceuticals sponsored a study of the AMPA receptor positive modulator (ampakine) CX516 in a 4 week placebo-controlled oral dose trial in adults with FXS (600 mg TID for 7 days, then 900 mg TID for 3 weeks) but were unable to show any associated improvement in memory, language, or attention/executive function.[97] Riluzole is approved by the U.S. Food and Drug Administration (FDA) for the treatment of amyotrophic lateral sclerosis and has shown beneficial clinical effects in depression and obsessive-compulsive disorder (OCD).[98] Together with its postulated mechanism of action through inhibition of glutamate release, blockade of the excitotoxic effects of glutamate, and enhancement of postsynaptic $GABA_A$ receptor function in the CNS, it has been considered a possible treatment for FXS. In an open-label 6 week clinical trial of riluzole (50 mg orally once daily for 1 week then increased to twice daily for 5 weeks) in 6 adults with FXS, no significant or consistent clinical improvement was demonstrated using the CGI-I subscale or other measure of OCD.[98]

mGluR5 Antagonists. The first trial of an mGluR5 antagonist was conducted with fenobam.[69] This trial, sponsored jointly by The FRAXA Research Foundation and the U.K.-based company Neuropharm, was a single dose, open-label study of 50−150 mg fenobam in 6 male and 6 female adults with FXS as an initial evaluation of safety and PK. No significant adverse reactions to fenobam were observed. Dose-dependent PK exposure was demonstrated in C_{max} that occurred around 180 min postdose and resembled normal volunteer PK, but showed high interindividual variability. Three subjects experienced sedation, while 9 of the patients were reported to exhibit calmed behavior with improvement in eye contact, ability to interact, anxiety, and/or motor overactivity. Changes in prepulse inhibition met the response criterion in 6 patients following fenobam. These are encouraging results for this means of glutamate receptor-based therapeutic intervention. Novartis Pharma AG, Hoffmann La-Roche, and Seaside Therapeutics have advanced mGluR5 antagonists into clinical trials. The Novartis compound has advanced into a larger trial in FXS patients based on data from a pilot study; these findings are discussed later in this review. The Roche study is a randomized, placebo-controlled, double-blind multiple ascending dose study with RO4917523 treatment over a 6 week period to evaluate safety and tolerability, PK and exploratory efficacy, and pharmacodynamic effects in adult patients with FXS (http://clinicaltrials.gov/ct2/show/NCT01015430?term=Fragile+X+syndrome&rank=15). Up to 100 patients are planned to be recruited for this study. Seaside Therapeutics has been progressing STX107 in single and multiple oral dose clinical trials in normal volunteers to assess safety and tolerability, PK, and exploratory pharmacodynamic effects (http://clinicaltrials.gov/ct2/show/NCT00965432?term=Fragile+X+syndrome&rank=24). Seaside anticipates moving into FXS patients with this agent during 2011.

Other Mechanism-Based Approaches. The peptide hormone oxytocin, a nonapeptide acting through a G-protein coupled receptor mechanism, is produced mainly by neurosecretory cells of the CNS and regulates a range of CNS functions.[99] It has been reported to have beneficial effects on CNS function, notably social behaviors.[99,100] Interestingly, it has been linked with therapeutic potential in autism spectrum disorders.[101] A randomized double-blind, placebo-controlled clinical trial with oxytocin (24 or 48 IU), in up to 12 adolescent male patients with genetically confirmed FXS, is in progress at Stanford University (http://clinicaltrials.gov/ct2/show/NCT01254045?term=Fragile+X+syndrome&rank=11). A variety of behavioral, cognitive, and physiological measures were employed to test for an efficacy signal, but to date no results have been reported.

Recent findings with the antibiotic minocycline have linked its matrix metalloproteinase inhibitory activity with the rescue of hippocampal dendritic spine development in *Fmr1*-deficient mice.[56] In an open-label 8 week clinical trial with 20 FXS patients, ages 13−32, randomly assigned to receive 100 or 200 mg of minocycline daily, statistically significant improvement in the Aberrant Behavior Checklist-Community Edition (ABC-C) Irritability subscale, Visual Analog Scale for behavior (VAS), and the CGI scale were reported.[102] Correction of the underlying neural defects that account for behavioral abnormalities in FXS by minocycline remains a possibility requiring further randomized, placebo-controlled clinical trial substantiation.

An open-label trial with lithium (0.8−1.2 mEq/L), reported to reduce mGluR-activated translation and reverse phenotypes in fragile X preclinical models, showed functional improvements in 15 individuals with FXS, aged 6−23, over a 2 month treatment period, suggesting further clinical evaluation is warranted.[103]

Functional cholinergic deficits may contribute to cognitive and behavioral dysfunction observed in FXS.[104] Enhancement of cholinergic function in the brain through administration of the acetylcholinesterase inhibitor donezepil (5 mg daily for 3 weeks, followed by 10 mg daily for 3 weeks) was associated with improvement on measures of cognition and behavior in an open-label trial involving 8 FXS patients 14 years of age and older.[104] A randomized, placebo-controlled trial with donezepil (2.5−10 mg for up to 12 weeks) in up to 50 individuals with FXS, ages 12−29 years, is currently in progress (http://clinicaltrials.gov/ct2/show/NCT00220584?term=-Fragile+X+syndrome&rank=8).

Recent Clinical Trial Directions. In the last year Novartis Pharma AG and Seaside Therapeutics have reported positive outcomes from blinded, placebo-controlled clinical trials in Fragile X patients. The Novartis study with their agent AFQ056[70] examined impact on the behavioral symptoms of FXS in a randomized, double blind, two-treatment, two-period, crossover design in 30 male adult patients, ages 18−35 years. A titration schedule was used for AFQ056 oral administration involving 50 mg twice daily on study days 1−4, 100 mg twice daily on days 5−8, 150 mg twice daily on days 9−20, 100 mg twice daily on days 21−24, and 50 mg twice daily on days 25−28. AFQ056 plasma concentrations were expected to reach steady state within 3−4 days. The compound had an approximate 20 h elimination half-life. Twenty-four of these patients experienced an adverse event, mostly mild to moderately severe fatigue or headache. No significant effects of treatment were detected on the primary outcome measure, the ABC-C score measured on day 19 or 20 of treatment. However, an exploratory analysis of the study data pointed to a statistically significant ($P < 0.001$) improvement in the ABC-C score for the subset of 7 patients who had full methylation of the *FMR1* promoter and no detectable *FMR1* mRNA. An eighth patient who had partial methylation but no detectable *FMR1* mRNA was not included in the subset analysis. Novartis initiated a further placebo-controlled multicenter clinical phase II/III 12 week treatment study with three oral dose levels of AFQ056 (25, 50, and 100 mg) in November 2010 to further assess safety and efficacy in a larger group of 160 adult FXS patients 18−45 years of age (http://clinicaltrials.gov/ct2/show/NCT01253629?term=Fragile+X+syndrome&rank=1). The primary outcome measure is change from baseline in behavioral symptoms of FXS using the ABC-C total score. Results are anticipated between late 2011 and early 2012.

Seaside Therapeutics, with their $GABA_B$ agonist, STX209, completed the largest randomized, placebo-controlled phase II study in FXS patients to date. Sixty-three patients, ages 6−40 years, with a Fragile X full mutation and a minimum severity on the ABC-I scale participated in a double-blind design trial to assess safety, tolerance, and efficacy across a broad range of behavioral and cognitive outcomes (http://www.seasidetherapeutics.com/). Dosing involved a flexible titration to the optimal titrated dose (1 mg up to 10 mg BID for subjects ≤11 years, and up to 10 mg TID for all other subjects), which was well tolerated. STX209 treatment showed a positive trend for the per protocol population of 54 patients across a range of global measures that included the CGI-I scale, the CGI-Severity (CGI-S), and investigator and caregiver treatment preference, but it did not meet its primary end point of improvement in the ABC-I scale relative to placebo. Interestingly, for 27 patients in the study with more severe impairment in sociability at baseline, STX209 treatment was associated with statistically significant improvements on all global measures. Two focused measures of social function, the ABC-Social Withdrawal and the Vineland Socialization domain score, also showed statistically significant improvement in that subset of patients. Seaside Therapeutics initiated larger registration clinical trials for STX209 in FXS during 2011.

CONCLUDING REMARKS

Both the Novartis and Seaside trials have provided insight into the challenges faced to achieve positive clinical trial outcomes in this syndromic disorder. The selection of appropriate indices of efficacy represents an area of uncertainty, as it is not possible to translate directly from animal responses to specific outcome measures in human clinical trials. Similarly, dose level and dosing duration cannot be predicted with certainty from the preclinical data. Furthermore, the requirement for full methylation status of the *FMR1* promoter and no detectable *FMR1* mRNA, if substantiated in large numbers of patients, may limit the treatable patient population generally for mGluR5 antagonists, but equally well it could speak to targeted drug therapy in this population. In conclusion, the recent clinical introduction of multiple compounds representing a variety of mechanistic approaches to the disorder represents an exciting opportunity to realize the mission of implementing effective treatments of ID.

ACKNOWLEDGMENT

The authors thank Drs., Paul Wang and Rebecca Hammond for helpful comments and edits to this review. We thank Dr. John Amedio for the STX209 graphic.

AUTHOR INFORMATION

Author Contributions

A.H. authored the FXS sections, T.O. the medicinal chemistry of mGluR5 antagonists and R.R. the therapeutic interventions.

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