Translating Molecular Advances in Fragile X Syndrome Into Therapy: A Review

Randi J. Hagerman, MD; Vincent Des-Portes, MD, PhD; Fabrizio Gasparini, PhD; Sébastien Jacquemont, MD; and Baltazar Gomez-Mancilla, MD, PhD

ABSTRACT

Fragile X syndrome is an inherited disease with cognitive, behavioral, and neurologic manifestations, resulting from a single genetic mutation. A variety of treatments that target individual symptoms of fragile X syndrome are currently utilized with limited efficacy. Research in animal models has resulted in the development of potential novel pharmacologic treatments that target the underlying molecular defect in fragile X syndrome, rather than the resultant symptoms. This review describes recent advances in our understanding of the molecular basis of fragile X syndrome and summarizes the ongoing clinical research programs.

J Clin Psychiatry 2014;75(4):e294–e307

Submitted: *August 5, 2013; accepted January 16, 2014 (doi:10.4088/JCP.13r08714).*
Corresponding author: *Baltazar Gomez-Mancilla, MD, PhD, Department of Neuroscience, Translational Medicine, Novartis Institutes of Biomedical Research, WSJ.210.25.5, CH-4056 Basel, Switzerland (baltazar.gomezmancilla@novartis.com).*

Fragile X syndrome is an X-linked disorder and is the most common form of inherited intellectual disability, estimated to affect between 1:2,500 and 1:4,000 individuals.[1–3] Males with fragile X syndrome display a broad spectrum of signs and symptoms that can be categorized as behavioral (including shyness, anxiety, social avoidance, aggression, attention-deficit/hyperactivity disorder [ADHD], and hand flapping), cognitive (including developmental delay and impairments of reasoning, executive function, visual memory, and visual-spatial processing), neurologic (including disrupted sleep patterns, obstructive sleep apnea, and epilepsy), and physical (including macroorchidism, prominent ears, long face, soft skin, and hyperextensible finger joints).[4–6] Approximately 30% of individuals with fragile X syndrome are diagnosed with autism, 60% have autism spectrum disorder,[7] 86% meet the criteria for an anxiety disorder,[8] and the intelligence quotient (IQ) range for fully affected males is typically between 20 and 70.[9] The X-linked nature of fragile X syndrome means that the phenotype of females with fragile X syndrome is generally less severe than that of males, although some females can be as severely affected as males. Approximately a quarter of females are defined as being intellectually disabled (IQ < 70), with the majority lying between the borderline and low-normal range of intelligence,[10] and 56% are reported to have anxiety.[11] The clinical presentation of fragile X syndrome varies markedly between individuals, which can make diagnosis difficult. Furthermore, awareness of the disease among parents, doctors, and teachers is poor, with many children being undiagnosed or misdiagnosed.[12,13]

GENETIC BASIS OF FRAGILE X SYNDROME

The wide variety of symptoms observed in fragile X syndrome originates from a loss of transcription of the fragile X mental retardation gene (*FMR1*), which is located at Xq27.3.[14] Translation of *FMR1* leads to expression of the active protein, fragile X mental retardation protein (FMRP). The 5′ promoter region of *FMR1* contains a CGG repeat that is variable in length and influences gene transcription and the subsequent translation of FMRP.[15] The likelihood of transmission of the defective gene increases with increasing maternal CGG repeat length.[16]

Individuals with a CGG repeat length of more than 200 repeats are described as having the full mutation of fragile X syndrome, and virtually all males inheriting the full mutation will develop the signs and symptoms of fragile X syndrome. Individuals with the full mutation exhibit *FMR1* promoter hypermethylation, which results in transcriptional silencing of *FMR1* and absence of FMRP[17] in a process thought to involve histone deacetylation and altered regulation of chromatin remodeling/modification.[18,19]

Individuals with 55–200 CGG repeats are said to have a premutation and are generally healthy, although they are at increased risk of developing an autism spectrum disorder,[20,21] late-onset

Clinical Points

- Most current treatments for fragile X syndrome, the most common cause of inherited intellectual disability, aim to treat individual symptoms of the disorder.
- Recent advances in our understanding of fragile X syndrome have allowed the development of pharmacologic agents that target the underlying molecular defect of the disease.
- Minocycline is the only targeted treatment for fragile X syndrome currently used in a clinical setting following initial positive findings from a controlled clinical trial. However, longer trials are required to establish the efficacy and safety of minocycline in patients with fragile X syndrome.
- Several other targeted agents are undergoing assessment in clinical trials, with the anticipation that they will lead to real improvements in the treatment and quality of life of patients and their families.

neurodegenerative disorder, fragile-X–associated tremor/ataxia syndrome (FXTAS),[22] mood or anxiety disorders,[23] immune-mediated disorders,[24] hypertension,[25] or seizures.[20] Females with the premutation are at risk of developing fragile-X–associated premature ovarian insufficiency (FXPOI) in adulthood.[26] In contrast to individuals with the full mutation, those with the premutation exhibit a 2-fold to 8-fold increase in *FMR1* mRNA levels, while FMRP levels are normal or only slightly reduced.[27,28] High levels of *FMR1* mRNA are thought to lead to an RNA toxic gain of function, which over time is thought to contribute to the development of FXTAS, FXPOI, and other symptoms of premutation involvement.[7,22,29]

Individuals with 45–55 CGG repeats are termed as having the intermediate allele or being in the "gray zone." These individuals are typically healthy, although females are at increased risk of FXPOI,[30] and there have been cases of FXTAS reported in individuals in the gray zone.[31] Individuals in the gray zone do not have fragile X syndrome; however, there may be an association with future generations developing fragile X syndrome.[32]

FRAGILE X MENTAL RETARDATION PROTEIN

Fragile X mental retardation protein is a 632-amino-acid protein produced by transcription of *FMR1*. It is primarily expressed in the brain and testes,[33] which is consistent with the clinical presentation of broad neurologic dysfunction and macroorchidism observed in fragile X syndrome. Brain regions showing particularly high expression of FMRP include the cerebellum, cortex, and hippocampus.[34,35] In individuals with fragile X syndrome, low levels of FMRP have been shown to correlate with intellectual disability, low IQ, impaired short-term memory, and a short attention span.[36–38]

In the brain, FMRP is primarily located in the dendrites,[39] soma, and postsynapse.[40] The 2 major functions of FMRP appear to be translational repression of a wide variety of proteins, including neurotransmitter receptor subtypes, cytoskeletal elements, ion channels, and adhesion molecules, and mRNA transport to the dendritic spines, a process during which mRNA transcription is also repressed.[41–43] The phosphorylation state of FMRP appears to be key to the translational repressive function of the protein, with phosphorylated FMRP being associated with stalled, non-translating polyribosomes, and unphosphorylated FMRP being associated with active, translating polyribosomes.[44]

Fragile X mental retardation protein has been shown to interact with approximately 4% of all mRNA in the brain.[45] Specific targets include microtubule-associated protein 1B (MAP1B), activity-regulated cytoskelaton-associated protein (Arc), striatal-enriched protein tyrosine phosphatase (STEP), myocyte enhancer factor 2 (MEF2), and synapse-associated protein 90/postsynaptic density protein 95 (PSD-95)–associated protein 3 (SAPAP3).[46–51] These proteins regulate several neurologic processes and pathways through which FMRP is thought to influence neurotransmitter release and synaptic plasticity, thus giving rise to the broad spectrum of symptoms observed when FMRP is absent in fragile X syndrome.[42,52]

THE *FMR1* KNOCKOUT MOUSE

Much of our understanding of the neurobiology of fragile X syndrome has been derived from study of the *FMR1* knockout mouse, which shares a number of anatomic and behavioral phenotypes with human fragile X syndrome. The cerebral cortex of the *FMR1* knockout mouse exhibits an increased density of postsynaptic dendrites that are long, thin, and tortuous.[53] Additional defects include olfactory learning deficits,[54] impaired motor learning[55] and memory function,[56] abnormal social behavior,[57,58] neuronal network hyperexcitability,[59] macroorchidism,[9] and increased protein synthesis.[60] The *FMR1* knockout mouse is also prone to epileptic seizures,[61] which are common in individuals with fragile X syndrome.[62]

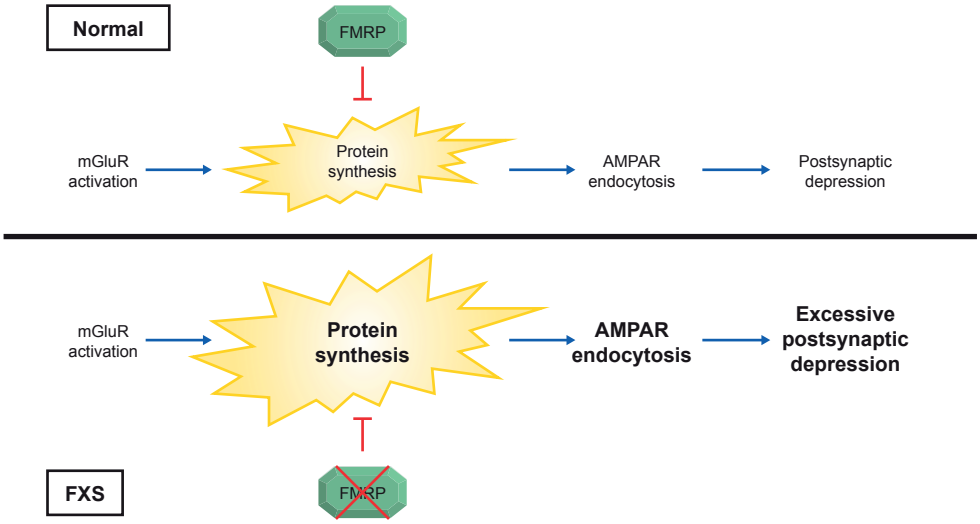
NEUROBIOLOGY OF FRAGILE X SYNDROME

Fragile X mental retardation protein has been shown to interact with aspects of the glutamate and γ-aminobutyric acid (GABA) signaling pathways, and alterations in these pathways have been demonstrated in the *FMR1* knockout mouse brain.[63,64] However, FMRP has also been implicated in the regulation of several other neurologic signaling pathways,[39] suggesting that the broad clinical profile observed in fragile X syndrome may be the product of simultaneous dysregulation of multiple signaling pathways.

Dysregulation of Metabotropic Glutamate Receptor Signaling in Fragile X Syndrome

Glutamate is the major excitatory neurotransmitter in the brain and signals through ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).[39] Ionotropic glutamate receptors include *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), which are fast-acting, ligand-gated ion channels.[65]

Figure 1. Absence of Fragile X Mental Retardation Protein (FMRP) Leads to Excessive Postsynaptic Depression



Abbreviations: AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, FXS = fragile X syndrome, mGluR = metabotropic glutamate receptor.

Of the 8 mGluRs, the group 1 mGluRs are of specific relevance to this review; these comprise mGluRs 1 and 5, which signal through G_q to activate phospholipase C.[66]

The most widely accepted mechanism by which absence of FMRP contributes to the fragile X syndrome phenotype is by enhancement of mGluR-mediated long-term depression, which is regulated though group 1 mGluRs.[66] Long-term depression is a key component of synaptic plasticity and is negatively regulated by FMRP; therefore, its absence results in disinhibition of long-term depression and altered synaptic plasticity.[63] Essentially, group 1 mGluR signaling leads to mRNA translation, protein expression, and subsequent internalization of postsynaptic AMPARs, thus reducing postsynaptic glutamate signaling for long periods.[67,68] Protein synthesis is not required for initiation of AMPAR endocytosis; however, it is essential for the observed decreases in postsynaptic AMPAR expression,[69] suggesting that proteins up-regulated by mGluR activation (termed *long-term depression proteins*) regulate trafficking of AMPARs.[70] Fragile X mental retardation protein is believed to play a central role in this process by inhibiting expression of long-term depression proteins, thus acting as a brake on AMPA internalization and negatively regulating long-term depression (Figure 1).[63]

Internalization of AMPARs is thought to involve Arc, MAP1B, and STEP. All 3 proteins are rapidly up-regulated following mGluR stimulation, and their knockdown results in inhibition of long-term depression and blockade of AMPAR internalization.[69–74] The scaffold protein Homer, which binds to mGluR,[75] is thought to play a key role by initiating protein synthesis through phosphoinositide 3-kinase (PI3K) enhancer[76] (Table 1).

Fragile X mental retardation protein is thought to inhibit expression of Arc and MAP1B through interactions with cytoplasmic FMRP-interacting protein 1 (CYFIP1), which is thought to dissociate from Arc and MAP1B following mGluR activation, promoting expression of these proteins and internalization of AMPARs.[84] Although a functional interaction between FMRP and STEP has not yet been demonstrated, activation of STEP is dependent on translation of preexisting mRNA in a mechanism similar to activation of Arc.[74] Fragile X mental retardation protein also regulates expression of SAPAP3 and PSD-95, which both function as scaffold proteins to link downstream signaling components to mGluR.[85,86] Activation of mGluR promotes dephosphorylation of FMRP, up-regulation of SAPAP3 and PSD-95, and inhibition of AMPAR internalization.[87,88] Finally, FMRP itself is rapidly degraded at the proteasome following activation of mGluR, which contributes to increases in the expression of FMRP-binding mRNAs.[72] A summary of mGluR-mediated long-term depression is presented in Figure 2.

Evidence Supporting Dysregulation of mGluR Signaling in Fragile X Syndrome

In 2004, Dolen et al[89] crossed the *FMR1* knockout mouse with mice heterozygous for the *Grm5* gene (which encodes mouse mGluR5). The resultant offspring lacked FMRP but also exhibited a 50% reduction in mGluR expression that was shown to rescue (or prevent) 7 of the 8 fragile X syndrome–related phenotypes assessed: audiogenic seizures were significantly reduced; increases in body weight, the number of dendritic spines in the visual cortex, and protein synthesis in the hippocampus were all reversed; excessive hippocampal long-term depression and abnormal experience-dependent plasticity of the visual cortex were corrected; and exaggerated inhibitory avoidance extinction (a mouse model for stress/fear-related learning and memory) was rescued. Macroorchidism was not reversed in this study, suggesting that testicular development may be, at least in part, regulated by other pathways.[90]

Table 1. Key Proteins Involved in mGluR-Mediated Long-Term Depression

Protein	Proposed Mechanism of Action	Evidence for Involvement in Long-Term Depression
Arc	A cytoskeletal protein rapidly expressed and transported to the synapses of active dendrites in response to neural activity[77,78] Associates with dynamin 2 and endophilin 2/3, known regulators of endocytosis[79] Synthesis in response to mGluR stimulation is thought to up-regulate and maintain AMPAR endocytosis[70,73]	Up-regulated following mGluR activation, and constitutive knockdown has been shown to block long-term depression and internalization of AMPAR[69,73]
MAP1B	Interacts with GRIP1, which stabilizes surface AMPAR subunits, an interaction that is strengthened following DHPG stimulation[80] This suggests that MAP1B promotes long-term depression by sequestering GRIP1 away from the synapse, destabilizing AMPAR and resulting in its internalization[70]	Expressed in dendrites and is known to interact with fragile X mental retardation protein[81,82] Treatment of hippocampal neurons with DHPG (a group 1 mGluR agonist) results in rapid up-regulation, and knockdown prevents DHPG-induced long-term depression [71,72]
STEP	Thought to induce AMPAR endocytosis by maintaining AMPAR in a tyrosine-dephosphorylated state[70]	Up-regulated following DHPG stimulation in hippocampal synaptoneurosomes[a] in a process that requires translation of preexisting RNA[74] Inhibition increases AMPAR surface expression, and knockdown inhibits DHPG-stimulated long-term depression[70]
Homer	Scaffold protein suggested to activate protein synthesis following mGluR activation Directly interacts with the C-terminus of mGluR,[75] and dimers have been shown to activate PIKE, which up-regulates the PI3K-mTOR pathway to initiate protein synthesis[76]	mGluR-Homer interactions are reduced in the *FMR1* knockout mouse, and blockade of mGluR5-Homer interactions prevents mGluR5 long-term depression in hippocampal slices[83]

[a]Experimental subcellular preparations containing components of both presynaptic and postsynaptic terminals.
Abbreviations: AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, Arc = activity-regulated cytoskeleton-associated protein, DHPG = dihydroxyphenylglycine, GRIP1 = glutamate receptor–interacting protein 1, MAP1B = microtubule-associated protein 1B, mGluR = metabotropic glutamate receptor, mTOR = mammalian target of rapamycin, PIKE = PI3K enhancer, PI3K = phosphoinositide 3-kinase, RNA = ribonucleic acid, STEP = striatal-enriched protein tyrosine phosphatase.

Other studies have focused on pharmacologic rescue of *FMR1* knockout mouse phenotypes, initially with 2-methyl-6-(phenylethynyl)pyridine (MPEP), the first prototypical potent negative allosteric modulator of mGluR5,[91] and later with improved pharmacologic agents specifically developed as mGluR5 antagonists. 2-Methyl-6-(phenylethynyl)pyridine reduced the likelihood of audiogenic seizures,[92] rescued deficits in prepulse inhibition of the startle response,[93] decreased mRNA granule expression,[94] reduced excessive hippocampal protein synthesis,[95] and increased the density of hippocampal dendritic cultures in the *FMR1* knockout mouse.[93] It was recently reported that chronic administration of the novel long-acting mGluR5 antagonist CTEP to *FMR1* knockout mice from 4 weeks of age restored several phenotypic deficits, including macroorchidism.[96]

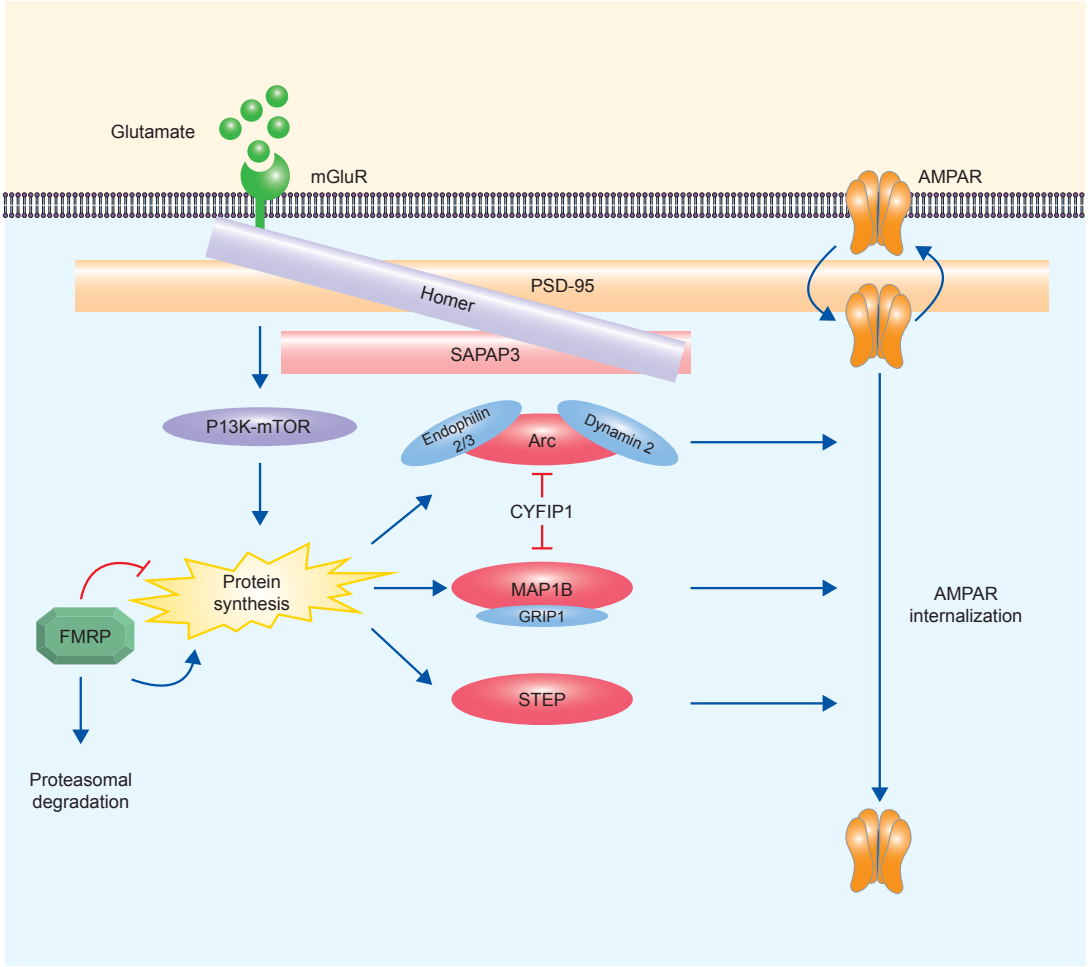
Dysregulation of GABA Signaling in Fragile X Syndrome

By signaling through $GABA_A$ receptors ($GABA_A$Rs) or $GABA_B$ receptors ($GABA_B$Rs), GABA is the main inhibitory neurotransmitter in the brain; altered GABA signaling is associated with epilepsy, anxiety, depression, and sleep disorders, all of which are common symptoms of fragile X syndrome.[52] $GABA_A$Rs are fast-acting ionotropic receptors that trigger Cl^- influx, whereas $GABA_B$Rs are metabotropic receptors that activate inwardly rectifying K^+ channels. The main function of $GABA_A$R is to hyperpolarize the postsynaptic membrane to decrease the likelihood of action potential propagation, a function also performed by $GABA_B$R; however, both receptors are also known to regulate presynaptic neurotransmitter release.[97]

Initial evidence for the involvement of GABA signaling in fragile X syndrome came from the finding that $GABA_A$R expression was reduced in the *FMR1* knockout mouse.[64,98] $GABA_A$R is a heteropentamer, with the subunit configuration enabling variation of signaling output in different brain regions.[52,97] Fragile X mental retardation protein binds to $GABA_A$R δ subunit mRNA,[64] and several studies have demonstrated considerable reductions in δ subunit mRNA, accompanied by slight reductions in α, β, and γ subunit mRNA, in the *FMR1* knockout mouse.[64,98] Other studies have also demonstrated reduced levels of α_1, β, and δ subunit protein expression in the *FMR1* knockout mouse.[99,100] Moreover, differential expression of a number of GABAergic signaling components has been demonstrated during development in the *FMR1* knockout mouse compared with controls,[99] along with electrophysiological dampening of the GABA system.[101] The potential of $GABA_A$R as a therapeutic target for fragile X syndrome was demonstrated recently by Heulens et al,[102] who reported reduced audiogenic seizures in *FMR1* knockout mice treated with ganaxolone, a selective positive allosteric modulator of $GABA_A$R.

Much research in GABA signaling in the *FMR1* knockout mouse has focused on the amygdala, a limbic region involved in the processing of emotion, fear, and other behavioral responses.[103] The *FMR1* knockout mouse amygdala has been shown to exhibit profound reductions in both the amplitude and frequency of fast inhibitory postsynaptic currents, decreased expression of glutamic acid decarboxylase (an enzyme that is the rate-limiting step in GABA synthesis), and impaired GABA release.[101] Furthermore, tonic GABA inhibition, a process thought to rely on expression of

Figure 2. mGluR-Mediated Long-Term Depression Can Be Influenced at Multiple Levels to Regulate Synaptic Plasticity



Abbreviations: AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, Arc = activity-regulated cytoskeleton-associated protein, CYFIP1 = cytoplasmic FMRP–interacting protein 1, FMRP = fragile X mental retardation protein, GRIP1 = glutamate receptor-interacting protein 1, MAP1B = microtubule-associated protein 1B, mGluR = metabotropic glutamate receptor, mTOR = mammalian target of rapamycin, PI3K = phosphoinositide 3-kinase, PSD-95 = postsynaptic density protein 95, SAPAP3 = synapse-associated protein 90/PSD-95–associated protein 3, STEP = striatal-enriched protein tyrosine phosphatase.

$GABA_AR$ subunits with high affinity for GABA (eg, δ and α_5[104,105]), is also reduced in the *FMR1* knockout mouse amygdala.[106]

$GABA_BR$ may also play a role in fragile X syndrome by inhibiting presynaptic glutamate release,[107] which may have inhibitory effects on postsynaptic mGluR5 and its downstream signaling. Reductions in $GABA_BR$ have been observed in the *FMR1* knockout mouse[108] and have been linked to autism, which is a common symptom of fragile X syndrome.[109] Furthermore, *FMR1* knockout mice treated with the $GABA_BR$ agonist baclofen exhibited reduced frequency of seizures in a process thought to be controlled by balancing of inhibitory GABA and stimulatory glutamate signaling.[110] Consistent with this, MPEP inhibition of glutamate signaling reduced the frequency of seizures in the *FMR1* knockout mouse,[92] while treating the *FMR1* knockout mouse with a combination of an mGluR agonist and a $GABA_BR$ antagonist induced seizures at doses that would not elicit seizures when administered separately.[110] Other examples of pharmacologic rescue of FMRP deficiency are reduced locomotor activity and hyperactivity in *FMR1* knockout mice treated with baclofen.[111]

Unanswered Questions and Additional Avenues of Research in the Neurobiology of Fragile X Syndrome

Although there is compelling evidence for the involvement of the mGluR and GABA pathways in the development of fragile X syndrome, it is unknown to what extent dysregulation of these pathways contributes to the overall fragile X syndrome phenotype. Fragile X mental retardation protein is widely expressed throughout the brain and has recently been proposed to regulate approximately half of the known genes associated with autism.[112] In light of this, it is not surprising that some observed phenotypes in the *FMR1* knockout mouse model cannot be explained simply by dysregulation of mGluR and GABA signaling. For example, absence of FMRP has been shown to regulate adult neurogenesis through novel pathways (possibly involving Wnt-canonical signaling),

which may contribute to learning and memory deficits in the *FMR1* knockout mouse.[113] With regard to the mGluR pathway, long-term potentiation in the cortex and amygdala (which partly depends on mGluR activation in wild-type mice) is attenuated in *FMR1* knockout mice, rather than being exaggerated as might be expected,[114,115] and once-daily injection of MPEP in *FMR1* knockout mice resulted in an increased density of immature dendritic spines in the somatosensory cortex[116] when they might have been expected to be reduced in number. Although most studies indicate a general dampening of GABA signaling in the *FMR1* knockout mouse brain,[52] increases in GABA output have been observed in some regions. For example, basal GABA inhibition was up-regulated in the striatum,[117] which may contribute to the frontostriatal dysfunction often observed in individuals with fragile X syndrome.[118] Other examples of the bidirectional effects on the GABA system include increases in the number of inhibitory GABA synapses within the CA1 region of the *FMR1* knockout hippocampus,[119] and decreased levels of glutamic acid decarboxylase in the amygdala[101] accompanied by increased glutamic acid decarboxylase levels in the cortex, hippocampus, and brainstem.[100]

Recent research has therefore focused on other pathways that may play a role in fragile X syndrome. Matrix metalloproteinase-9 (MMP-9) is a regulator of extracellular protein degradation and is up-regulated in fragile X syndrome.[120] The antibiotic minocycline inhibits MMP-9 and has been shown to promote expression of mature dendritic spines, improve behavioral performance,[121] and restore mating vocalization deficits[122] in the *FMR1* knockout mouse. Oxidative stress has been proposed to contribute to fragile X syndrome, and melatonin (an endocrine regulator of the circadian rhythm that also has antioxidative properties) was found to normalize glutathione and lipid peroxidation levels (markers of oxidative stress) in the *FMR1* knockout mouse brain.[123] Another antioxidant, α-tocopherol, was shown to reverse free-radical overproduction and rescue behavioral and learning deficits.[124] Lithium, used successfully to treat mood disorders through inhibition of glycogen synthase kinase-3β, has been shown to normalize extracellular signal-regulated kinase levels, restore normal mGluR-dependent long-term depression, and improve behavioral deficits in the *FMR1* knockout mouse,[125] while antagonism of NMDA receptors by memantine has been shown to rescue immature dendritic morphology and promote synapse formation in the *FMR1* knockout mouse.[126] As described below, many of these agents are now being tested in humans, with promising results.

THERAPEUTIC INTERVENTIONS FOR FRAGILE X SYNDROME

Current Therapies for Fragile X Syndrome

Current pharmacologic interventions for fragile X syndrome aim to alleviate individual symptoms of fragile X syndrome such as ADHD, anxiety, and aggression. Treatments commonly used in individuals with fragile X syndrome include stimulants, antipsychotics, α_2 agonists, and selective serotonin reuptake inhibitors (SSRIs); current treatment typically includes a stimulant and an SSRI.[127] While these treatments can offer some benefit to the patient, each generally tackles only an individual symptom of the disease, although they may be utilized in combination with medications targeting the underlying molecular defects of fragile X syndrome. There is little published evidence on the efficacy of these treatments for fragile X syndrome.[128] Only 1 controlled trial (published in 1998)[129] has demonstrated the efficacy of dopamine agonists (methylphenidate and dextroamphetamine) in fragile X syndrome, leading to the common use of stimulants in individuals aged 5 years and older.[127]

Instead of targeting individual symptoms, recent developments in our understanding of the molecular basis of fragile X syndrome have led to the development of several therapeutic agents that target the underlying mechanisms of the disease (Table 2 and Figure 3).

Agents Targeting the mGluR Pathway

In a phase 2 trial,[131] 30 males with fragile X syndrome were treated with either the mGluR5 antagonist AFQ056/mavoglurant or placebo for 28 days. No significant improvements in the primary end point of the Aberrant Behavior Checklist-Community Edition (ABC-C) total score were observed within the whole study population, although an improvement was observed on the Repetitive Behavior Scale-Revised. However, exploratory analysis on the 7 individuals exhibiting full promoter methylation of the *FMR1* gene revealed significant improvements in ABC-C scores, the Clinical Global Impressions (CGI) scale, and most other secondary end points studied. There are 2 ongoing phase 2b studies assessing the safety and efficacy of AFQ056/mavoglurant: 1 in adults and another in adolescents aged 12–17 years.

Other mGluR antagonists being tested in individuals with fragile X syndrome include STX107 and RO4917523/basimglurant (also designated RG7090). STX107 is a novel and potent mGluR5 antagonist that has undergone phase 1 safety testing in adults. A phase 2 study was subsequently initiated to assess tolerability and pharmacokinetic outcomes. RO4917523 is an mGluR5 antagonist in development to treat fragile X syndrome and has demonstrated a favorable safety profile in an initial phase 2 trial in 40 adults with fragile X syndrome. Two further phase 2 studies—1 on safety in pediatric use and the second on efficacy and safety in adults—are recruiting at the time of this article's development. Another mGluR antagonist, fenobam, was tested in a pilot open-label trial[132] of 12 individuals with fragile X syndrome, with positive results regarding improvement in prepulse inhibition and no safety concerns. A subsequent phase 1 pharmacokinetic and safety trial in 32 healthy adults is currently recruiting.

Agents Targeting GABA Pathways

Arbaclofen (STX209) is the active enantiomer of the $GABA_BR$ agonist baclofen that reduces hyperactivity,

Table 2. Therapies Targeting the Underlying Cause of Fragile X Syndrome (FXS) That Are Undergoing Clinical Trials

Agent	Clinical Evidence in FXS	Status of Planned or Ongoing Trials
mGluR5 Antagonists		
AFQ056/ mavoglurant	Phase 2, randomized, double-blind, placebo-controlled study[131] 30 males aged 18–35 years with FXS Significant difference between AFQ056/mavoglurant and placebo for RBS-R overall but not ABC-C (primary outcome) or other secondary outcomes Subgroup analysis of individuals with fully methylated *FMR1* promoter showed significant differences between AFQ056/ mavoglurant and placebo for ABC, CGI-Improvement, CGI efficacy, RBS-R, SRS, VAS, but not VABS, as well as measures of hyperactivity, inappropriate speech, and stereotypic behavior	Ongoing: 2 phase 2b, double-blind, randomized, placebo-controlled trials in individuals with FXS to assess the efficacy of 3 AFQ056/ mavoglurant doses on behavioral symptoms One in adolescents (12–17 years; NCT01433354) One in adults (18–45 years; NCT01348087)
STX107	None	Planned: phase 2, randomized, double-blind, placebo-controlled study of a single dose of STX107 (10 mg or 30 mg) in 16 adults with FXS (ClinicalTrials.gov identifier: NCT01325740) Recruitment suspended pending further evaluation
RO4917523 (RG7090)	Phase 2 trial in 40 individuals with FXS reported favorable safety profile for RG7090 (ClinicalTrials.gov identifier: NCT01015430)	Recruiting: phase 2, randomized, double-blind, placebo-controlled efficacy and safety study of 180 individuals with FXS aged 16–50 years (ClinicalTrials.gov identifier: NCT01517698) Individuals will be treated with RG7090 0.5 mg or 1.5 mg over 12 weeks Recruiting: phase 2, randomized, double-blind, placebo-controlled safety and preliminary efficacy study of 45 individuals with FXS aged 5–13 years (ClinicalTrials.gov identifier: NCT01750957) Individuals will be treated with 2 doses (not specified) of RG7090 over 12 weeks
Fenobam (NPL2009)	Open-label, single-dose pilot study showed that 6 of 12 individuals with FXS had a >20% improvement in prepulse inhibition but no improvements in Continuous Performance Test[132]	Recruiting: phase 1, blinded, randomized, placebo-controlled, pharmacokinetic study of fenobam 50, 100, or 150 mg in healthy adult volunteers
$GABA_BR$ agonist		
Arbaclofen (STX209)	Phase 2 randomized, double-blind, placebo-controlled trial of 63 individuals with FXS. No improvement in ABC-irritability subscale (the primary end point); however, improvements on the VAS ratings of parent-nominated behaviors and ABC-social avoidance subscale were noted[133]	Study terminated: phase 2, open-label, efficacy and safety extension study in 45 children and adults with FXS (ClinicalTrials.gov identifier: NCT01013480) Study terminated: phase 3 open-label trial to evaluate efficacy, safety, and pharmacokinetics in 357 individuals with FXS aged 5–50 years (ClinicalTrials.gov identifier: NCT01555333) Two phase 3, double-blind, placebo-controlled trials for the treatment of social withdrawal in individuals with FXS Completed: adolescents and adults, aged 12–50 years (n = 125) (ClinicalTrials.gov identifier: NCT01282268) Completed: children, aged 5–11 years (n = 172) (ClinicalTrials.gov identifier: NCT01325220) Primary end point for both trials is the ABC-lethargy social withdrawal subscale
$GABA_AR$ agonist		
Ganaxolone	None	Recruiting: phase 2, double-blind, controlled trial in up to 60 children with FXS, aged 6–17 years, to assess safety, tolerability, and efficacy (ClinicalTrials.gov identifier: NCT01725152)
Acamprosate	Retrospective review of medical records for 3 individuals with FXS and autism receiving acamprosate for 16–28 weeks showed a global clinical benefit as rated on the CGI-Improvement scale score for all individuals[134] Open-label, uncontrolled study in 7 individuals with FXS and autism and 6 individuals with autism alone[135] Significant improvements from baseline in all outcome measures were observed (CGI-Irritability, CGI-Severity, ABC scales and VAS) 10 of 13 individuals were reported to be clinical responders	Recruiting: phase 2/3 double-blind, placebo-controlled, proof-of-concept study in 48 adolescents, aged 5–18 years (ClinicalTrials.gov identifier: NCT01911455) Completed: phase 3, open-label, uncontrolled efficacy study in individuals aged 5–17 years with FXS (ClinicalTrials.gov identifier: NCT01300923)

(continued)

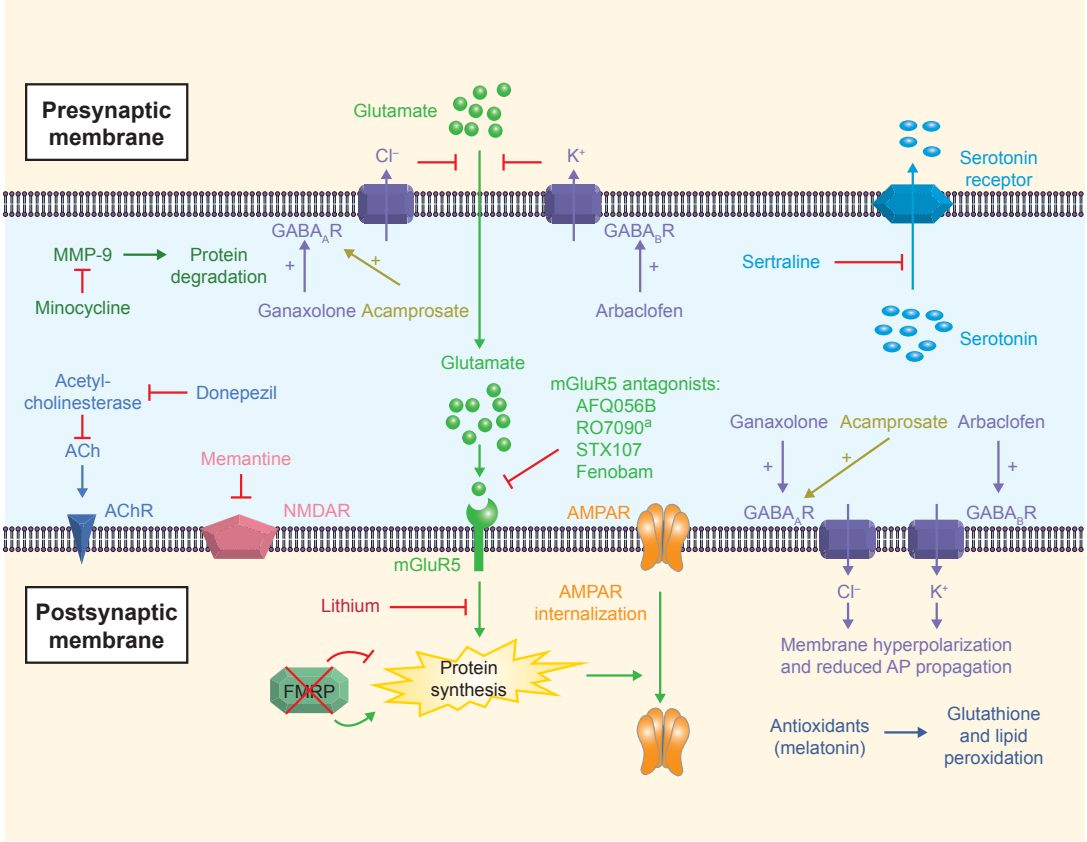
Table 2 (continued). Therapies Targeting the Underlying Cause of Fragile X Syndrome (FXS) That Are Undergoing Clinical Trials

Agent	Clinical Evidence in FXS	Status of Planned or Ongoing Trials
Other signaling pathways		
Minocycline	Survey of 50 individuals who received minocycline for at least 2 weeks reported an improvement in language and behavior areas according to parents' feedback[136] An open-label, 8-week trial, in 20 individuals aged 13–32 years treated with minocycline 100–200 mg for 8 weeks reported significant improvements in ABC-C irritability subscale and VABS[137] Adverse events were generally mild A double-blind, placebo-controlled trial in 55 children and adolescents, aged 3.5–16 years, showed positive results in the CGI-Improvement scale score and in mood and anxiety problems as measured by a VAS[138]	None ongoing
Lithium	Open-label study in 15 individuals with FXS showed that 2 months of treatment with lithium significantly improved total ABC-C, VAS, CGI, VABS, and RBANS list learning scales[139] Lithium improved anxiety, tantrums, mood swings, and aggression; and caregiver ratings indicated improvements in hyperactivity and inappropriate speech	None planned
Melatonin	A 4-week, randomized, controlled, phase 2 trial of 12 children with autism and FXS showed significant improvements in mean sleep duration, mean sleep-onset latency, and mean sleep-onset time[140]	None planned
Donepezil	Open-label pilot study of 8 individuals with FXS, aged 14–44 years. Subjects demonstrated improvements in ABC total, irritability, and hyperactivity scores and contingency naming task scores (a measure of working memory and mental flexibility)[141]	Recruiting: phase 2 RCT of 50 individuals with FXS, aged 12–29 years, receiving donepezil 2.5–10 mg/d for 12 weeks (ClinicalTrials.gov identifier: NCT01120626)
Sertraline	Open-label study of 11 children with FXS. Sertraline improved receptive and expressive language development in comparison to controls[142]	Recruiting: phase 2, double crossover RCT of 72 children, aged 24–68 months, receiving sertraline 2.5–5 mg/d for 6 months (ClinicalTrials.gov identifier: NCT01474746)
Lovastatin	Open-label case reports (R. J. H., unpublished data, 2013)	Planned controlled trial
Memantine	Retrospective review of medical records for 6 individuals with FXS and repetitive developmental disorders showed no significant improvement on CGI-Irritability scales or other symptom-specific rating scales[143]	Recruiting: phase 2, double-blind, placebo-controlled, dose escalation (5–20 mg) study for symptoms of FXTAS in 180 individuals aged > 30 years (ClinicalTrials.gov identifier: NCT00584948)
Riluzole	Open-label study in 6 individuals with FXS showed no significant improvement in the CGI-Irritability subscale[144]	None planned
CX516	Phase 2, randomized, double-blind, placebo-controlled, 4-week trial in 49 individuals with FXS showed no significant improvement in cognitive and behavioral outcome measures compared with placebo[145]	None planned
Oxytocin	A double-blind placebo controlled study of 10 male adolescents (13–24 y) with FXS receiving a single dose of 24 or 48 IU of oxytocin or placebo, over 3 consecutive wk (1 wk apart). Eye contact was significantly improved with the 24-IU dose compared with placebo, and salivary cortisol levels were significantly reduced with the 48-IU dose compared with placebo[150]	None planned

Abbreviations: ABC = Aberrant Behavior Checklist, ABC-C = Aberrant Behavior Checklist-Community, CGI = Clinical Global Impressions, FXS = fragile X syndrome, FXTAS = fragile-X-associated tremor/ataxia syndrome, $GABA_AR$ = γ-aminobutyric acid-A receptor, $GABA_BR$ = γ-aminobutyric acid-B receptor, IU = international unit, LTD = long-term depression, mGluR5 = metabotropic glutamate receptor 5, RBANS = Repeatable Battery for the Assessment of Neuropsychological Status, RBS-R = Repetitive Behavior Scale-Revised, RCT = randomized controlled trial, SRS = Social Responsiveness Scale, VABS = visual analog scale of behavior, VAS = visual analog scale.

the frequency of seizures,[110,111] and excessive protein production and rescues excess spine production in *FMR1* knockout mice.[146] Arbaclofen has been studied in a phase 2 randomized controlled trial involving 63 individuals with fragile X syndrome. It was well tolerated and produced modest improvements in behavior and social avoidance scores, particularly in those with autism or social withdrawal.[133] Further phase 2 and phase 3 studies have investigated behavioral outcomes, including social withdrawal and irritability as well as safety and tolerability in adolescents and adults with fragile X syndrome. The phase 3 randomized double-blind study on social withdrawal has been completed, and the 2 ongoing open-label pharmacokinetic and safety studies have been discontinued; to date, the results of these studies have not been reported.

Figure 3. Therapeutic Interventions That Aim to Target the Molecular Pathology of Fragile X Syndrome



[a]RO7090 was previously called RO4917523.

Abbreviations: ACh = acetylcholine, AChR = acetylcholine receptor, AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, AP = action potential, FMRP = fragile X mental retardation protein, GABA = γ-aminobutyric acid, GABA$_A$R = GABA$_A$ receptor, GABA$_B$R = GABA$_B$ receptor, mGluR5 = metabotropic glutamate receptor 5, MMP-9 = matrix metalloproteinase-9, NMDAR = *N*-methyl-D-aspartate receptor.

Ganaxolone is a synthetic analog of the neuroactive steroid allopregnanolone, a positive allosteric modulator of GABA$_A$R that has been shown to reduce the frequency of audiogenic seizures in the *FMR1* knockout mouse.[102] Clinical trials have shown that ganaxolone is effective at reducing seizure frequency in adults and children with epilepsy,[147,148] and trials are currently taking place in children with fragile X syndrome. Benzodiazepines have also been used successfully in those with fragile X syndrome for acute management of anxiety-provoking situations, such as a dental appointment or an airplane flight, or for treatment of aggression, but sedation is common, interfering with chronic use of these agents.

Agents Targeting Other Pathways

Agents targeting several other pathways have been tested in individuals with fragile X syndrome, although many of these are limited to nonplacebo controlled trials. Care must therefore be taken when interpreting these findings.

Acamprosate is a drug approved for use in adults with alcohol dependence that is thought to inhibit glutamate signaling and stimulate GABA$_A$R signaling. An initial retrospective study[134] of 3 individuals with fragile X syndrome and autism showed improvements in the CGI scale following administration of acamprosate for 16–28 weeks. A phase 2, open-label trial[135] in individuals with fragile X syndrome and autism subsequently showed improvements in CGI, ABC-C, and SRS scores. A phase 2/3 trial on efficacy is ongoing.

In a survey of 50 families with a child with fragile X syndrome, two-thirds reported that 2 weeks of treatment with minocycline (a tetracycline antibiotic) improved behavior, language, and attention levels.[136] Similar results were observed in an 8-week, open-label extension study[137] in which minocycline was also shown to be well tolerated. Of the 19 families who completed the extension study, 18 decided to continue therapy for a further year based on their perceptions of improved behavior. A controlled crossover trial[138] of 55 children with fragile X syndrome, aged 3.5–16 years, was recently completed, with significant improvement in the CGI score and in mood and anxiety problems as measured by the visual analog scale (VAS). Minocycline is available by prescription and is currently used clinically to treat children and adults with fragile X syndrome.

Lithium has long been used to treat mood disorders.[39] In an open-label trial[149] of 15 individuals with fragile X syndrome, 2 months of treatment with lithium improved

ABC-C, VAS, and CGI-Improvement scores,[139] results that were maintained in the 11 individuals who continued therapy up to 1 year.

Other agents showing therapeutic promise include melatonin, which may improve sleep problems[140]; donepezil, which has been shown to improve behavior and working memory[141]; and sertraline, which improved receptive and expressive language in children with fragile X syndrome.[142] Further trials of donepezil and sertraline are recruiting and, although no further trials are planned for melatonin, a phase 2 trial of the antioxidants ascorbic acid and α-tocopherol is ongoing, demonstrating the promise of targeting oxidative stress in individuals with fragile X syndrome. No significant improvements were observed in individuals receiving memantine in a retrospective review[143] of 6 individuals with fragile X syndrome; however, a dose-escalation trial is currently recruiting. Riluzole[144] (an inhibitor of glutamate release and potentiator of $GABA_AR$ activity) and CX516[145] (an AMPAR allosteric positive modulator) were both ineffective in small trials. Oxytocin has been tested versus placebo in a randomized controlled trial in which 8 males (age, mean = 21.3 years; range, 13–28) received a single daily dose of oxytocin for 7 days.[150] These individuals then underwent a structured social challenge with monitoring of eye contact, cardiac measures, and saliva cortisol. Oxytocin improved eye contact frequency with the 24 IU dose compared with placebo, whereas the saliva cortisol level decreased significantly versus placebo with the 48 IU dose. However, there was no effect on the heart rate level, heart rate variability, or respiratory sinus arrhythmia.

Timing of Treatment Initiation

From a clinical perspective, it is generally assumed that the earlier the intervention, the better the outcome. In autism, beneficial outcomes have been observed in individuals receiving early behavioral interventions.[151] An enriched environment has been shown to improve synaptic connectivity in animal models of fragile X syndrome,[152] and intensive early initiation with a combination of targeted treatments and educational interventions has been very helpful in case studies of young children with fragile X syndrome,[153] giving hope of reversing intellectual disability and behavior problems for this syndrome. Although optimal outcomes may be achieved through early intervention, studies in animal models show that targeted treatment during adulthood is able to reverse many aspects of the fragile X syndrome phenotype.[96] Benefits have also been observed in adults treated with arbaclofen[133] and AFQ056/mavoglurant,[131] although, in the arbaclofen trial, no significant age-related effects were found on any of the outcome measures.[133]

CHALLENGES

Although many of the targeted treatments described here have demonstrated significant efficacy across multiple features of fragile X syndrome in the knockout mouse model, none has so far achieved the same effect in humans. In most studies, benefits have been demonstrated in only a subgroup of individuals with fragile X syndrome, such as those with full methylation in the AFQ056/mavoglurant study[131] and those with social deficits or autism spectrum disorder in the arbaclofen study.[133] Although some individuals demonstrate an excellent response to minocycline, others with a mild response or no response may exhibit adverse events such as hyperarousal, preventing widespread minocycline use.[138] This heterogeneity is most likely due to background genetic effects or environmental differences; detailed study is required to profile these among individuals.

Appropriate tools are required to assess outcomes, which will hopefully improve with these new targeted treatments. For example, quantitative event–related potential studies have recently been employed to demonstrate significant improvements in brain information processing in children with fragile X syndrome treated with minocycline,[154] and an expressive language outcome measure (sensitive to improvements in development rather than only in behavior) has recently been developed[145,146]; it is hoped that these measures will demonstrate improvements in language and cognition in individuals with fragile X syndrome. In addition, biochemical markers, such as the rate of extracellular signal-regulated kinase phosphorylation (which improved with lithium treatment)[139] or the down-regulation of MMP-9 (used alongside minocycline treatment of children with fragile X syndrome)[120] should be utilized more frequently to provide a direct measure of biochemical improvements with specific targeted treatments.

Most studies performed in individuals (particularly adults) with fragile X syndrome occur over 2–3 months, which is likely to be too short for significant improvements (eg, in cognition) to be detected. New medications must be approved for adults and adolescents before approvals are given to include children in such trials, although children with fragile X syndrome are likely to exhibit more rapid and beneficial improvements in response to targeted treatments than adults.[142,153] Long-term controlled trials are typically not carried out, owing to the expense and level of patient cooperation required, although open-label studies are usually long term and are more likely to show cognitive benefits. A learning paradigm to assess the benefits regarding cognition in long-term studies would be useful to understand improvements beyond those in behavior. Such learning paradigms could include a digital program to improve ADHD symptoms, executive function training, or intensive reading or numeracy programs. Additionally, the use of multiple medications, such as an mGluR5 antagonist combined with a GABA agonist or minocycline may prove to be more beneficial than either medication alone, particularly if they involve different dysfunctional pathways resulting from the lack of FMRP.

CONCLUSION

An array of animal and human studies has shown that FMRP regulates several neurologic signaling pathways,

providing an explanation of how the genetic defect may cause the wide range of cognitive and behavioral defects observed in fragile X syndrome. This research has also stimulated the development of new therapeutic agents that target the underlying mechanistic abnormalities of the disease. While these new agents are an advance on existing symptomatic treatments for fragile X syndrome, it is essential that they are tested in appropriate randomized, controlled, clinical settings to assess their effectiveness and safety.

Although our understanding of fragile X syndrome has increased considerably in recent years, awareness of the disease remains poor among the general public and clinicians alike. The potential availability of treatments for fragile X syndrome that improve cognition, prevent the development of social deficits, and reduce caregiver burden means that identification of individuals with fragile X syndrome who may benefit from treatment is a priority. Clear education strategies are required so that early signs of the disease are identified, while newborn screening tools have been developed for early identification of individuals with fragile X syndrome.[155] Some targeted treatments can be initiated in early childhood, which is likely to be the optimal time for reversing the intellectual deficits of fragile X syndrome.

Drug names: acamprosate (Campral and others), baclofen (Gablofen, Lioresal, and others), donepezil (Aricept and others), lithium (Lithobid and others), lovastatin (Altoprev and others), memantine (Namenda), methylphenidate (Daytrana, Ritalin, and others), minocycline (Dynacin, Minocin, and others), oxytocin (Pitocin and others), riluzole (Rilutek and others), sertraline (Zoloft and others).

Author affiliations: Department of Pediatrics and MIND Institute, School of Medicine, University of California, Davis (Dr Hagerman); Reference Center for Intellectual Disability, HCL, Bron; L2C2 CNRS UMR 5304, Lyon 1 University, Lyon (Dr Des-Portes), France; Novartis Institutes for BioMedical Research, Basel, Switzerland (Drs Gasparini and Gomez-Mancilla); and Central Hospital, University of Vaudois, Lausanne, Switzerland (Dr Jacquemont).

Author contributions: All authors contributed to the development and writing of the manuscript, and approved the final article for publication.

Potential conflicts of interest: **Drs Gasparini** and **Gomez-Mancilla** are employees of Novartis Pharma AG. **Dr Hagerman** has received funding from Roche, Novartis, Curemark, Forest, and Seaside Therapeutics to carry out treatment trials in fragile X syndrome or autism, and has consulted with Novartis, Roche, and Genentech regarding fragile X syndrome treatment studies. **Dr Des-Portes** has received funding from Roche and Novartis to carry out treatment trials in fragile X syndrome. **Dr Jacquemont** has received funding from Novartis to carry out treatment trials in fragile X syndrome and has served as a consultant, received honoraria, and served on speakers or advisory boards for Novartis.

Funding/support: This review was funded by Novartis Pharma AG, Basel, Switzerland.

Role of sponsor: Novartis was involved in all stages of the study, including study design, data collection, management, analysis, and interpretation, as well as preparation, review, and approval of the manuscript. Novartis also provided funding to Oxford PharmaGenesis for help in developing this article.

Acknowledgment: The authors thank Adam Giles, PhD, from Oxford PharmaGenesis Ltd for editorial support in producing this review. Dr Giles reports no additional conflicts of interest.

REFERENCES

1. Hagerman PJ. The fragile X prevalence paradox. *J Med Genet*. 2008;45(8):498–499.
2. Coffee B, Keith K, Albizua I, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *Am J Hum Genet*. 2009;85(4):503–514.
3. Fernandez-Carvajal I, Walichiewicz P, Xiaosen X, et al. Screening for expanded alleles of the FMR1 gene in blood spots from newborn males in a Spanish population. *J Mol Diagn*. 2009;11(4):324–329.
4. Gross C, Berry-Kravis EM, Bassell GJ. Therapeutic strategies in fragile X syndrome: dysregulated mGluR signaling and beyond. *Neuropsychopharmacology*. 2012;37(1):178–195.
5. Hampson DR, Adusei DC, Pacey LK. The neurochemical basis for the treatment of autism spectrum disorders and fragile X syndrome. *Biochem Pharmacol*. 2011;81(9):1078–1086.
6. McLennan Y, Polussa J, Tassone F, et al. Fragile X syndrome. *Curr Genomics*. 2011;12(3):216–224.
7. Hagerman R, Au J, Hagerman P. FMR1 premutation and full mutation molecular mechanisms related to autism. *J Neurodev Disord*. 2011;3(3):211–224.
8. Cordeiro L, Ballinger E, Hagerman R, et al. Clinical assessment of *DSM-IV* anxiety disorders in fragile X syndrome: prevalence and characterization. *J Neurodev Disord*. 2011;3(1):57–67.
9. Fisch GS, Simensen RJ, Schroer RJ. Longitudinal changes in cognitive and adaptive behavior scores in children and adolescents with the fragile X mutation or autism. *J Autism Dev Disord*. 2002;32(2):107–114.
10. Hagerman RJ, Jackson C, Amiri K, et al. Girls with fragile X syndrome: physical and neurocognitive status and outcome. *Pediatrics*. 1992;89(3):395–400.
11. Bailey DB Jr, Raspa M, Olmsted M, et al. Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. *Am J Med Genet A*. 2008;146A(16):2060–2069.
12. Bailey DB Jr, Raspa M, Bishop E, et al. No change in the age of diagnosis for fragile X syndrome: findings from a national parent survey. *Pediatrics*. 2009;124(2):527–533.
13. Kemper AR, Bailey DB Jr. Pediatricians' knowledge of and attitudes toward fragile X syndrome screening. *Acad Pediatr*. 2009;9(2):114–117.
14. Verkerk AJ, Pieretti M, Sutcliffe JS, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991;65(5):905–914.
15. Kirchgessner CU, Warren ST, Willard HF. X inactivation of the FMR1 fragile X mental retardation gene. *J Med Genet*. 1995;32(12):925–929.
16. McConkie-Rosell A, Abrams L, Finucane B, et al. Recommendations from multi-disciplinary focus groups on cascade testing and genetic counseling for fragile X-associated disorders. *J Genet Couns*. 2007;16(5):593–606.
17. Godler DE, Tassone F, Loesch DZ, et al. Methylation of novel markers of fragile X alleles is inversely correlated with FMRP expression and FMR1 activation ratio. *Hum Mol Genet*. 2010;19(8):1618–1632.
18. Kumari D, Usdin K. The distribution of repressive histone modifications on silenced FMR1 alleles provides clues to the mechanism of gene silencing in fragile X syndrome. *Hum Mol Genet*. 2010;19(23):4634–4642.
19. Pietrobono R, Tabolacci E, Zalfa F, et al. Molecular dissection of the events leading to inactivation of the FMR1 gene. *Hum Mol Genet*. 2005;14(2):267–277.
20. Chonchaiya W, Au J, Schneider A, et al. Increased prevalence of seizures in boys who were probands with the FMR1 premutation and co-morbid autism spectrum disorder. *Hum Genet*. 2012;131(4):581–589.
21. Farzin F, Perry H, Hessl D, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr*. 2006;27(suppl 2):S137–S144.
22. Garcia-Arocena D, Hagerman PJ. Advances in understanding the molecular basis of FXTAS. *Hum Mol Genet*. 2010;19(R1):R83–R89.
23. Bourgeois JA, Seritan AL, Casillas EM, et al. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. *J Clin Psychiatry*. 2011;72(2):175–182.
24. Winarni TI, Chonchaiya W, Sumekar TA, et al. Immune-mediated disorders among women carriers of fragile X premutation alleles. *Am J Med Genet A*. 2012;158A(10):2473–2481.
25. Hamlin AA, Sukharev D, Campos L, et al. Hypertension in FMR1 premutation males with and without fragile X-associated tremor/ataxia syndrome (FXTAS). *Am J Med Genet A*. 2012;158A(6):1304–1309.
26. Sullivan SD, Welt C, Sherman S. FMR1 and the continuum of primary ovarian insufficiency. *Semin Reprod Med*. 2011;29(4):299–307.
27. Allen EG, He W, Yadav-Shah M, et al. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. *Hum Genet*. 2004;114(5):439–447.
28. Tassone F, Hagerman RJ, Taylor AK, et al. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet*. 2000;66(1):6–15.
29. Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. *Lancet Neurol*. 2013;12(8):786–798.
30. Bodega B, Bione S, Dalprà L, et al. Influence of intermediate and uninterrupted FMR1 CGG expansions in premature ovarian failure

manifestation. *Hum Reprod*. 2006;21(4):952–957.
31. Hall D, Tassone F, Klepitskaya O, et al. Fragile X-associated tremor ataxia syndrome in FMR1 gray zone allele carriers. *Mov Disord*. 2012;27(2):296–300.
32. Fernandez-Carvajal I, Lopez Posadas B, Pan R, et al. Expansion of an FMR1 grey-zone allele to a full mutation in two generations. *J Mol Diagn*. 2009;11(4):306–310.
33. Devys D, Lutz Y, Rouyer N, et al. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat Genet*. 1993;4(4):335–340.
34. Abitbol M, Menini C, Delezoide AL, et al. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. *Nat Genet*. 1993;4(2):147–153.
35. Hinds HL, Ashley CT, Sutcliffe JS, et al. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nat Genet*. 1993;3(1):36–43.
36. Dyer-Friedman J, Glaser B, Hessl D, et al. Genetic and environmental influences on the cognitive outcomes of children with fragile X syndrome. *J Am Acad Child Adolesc Psychiatry*. 2002;41(3):237–244.
37. Loesch DZ, Huggins RM, Hagerman RJ. Phenotypic variation and FMRP levels in fragile X. *Ment Retard Dev Disabil Res Rev*. 2004;10(1):31–41.
38. Tassone F, Hagerman RJ, Iklé DN, et al. FMRP expression as a potential prognostic indicator in fragile X syndrome. *Am J Med Genet*. 1999;84(3):250–261.
39. Levenga J, de Vrij FM, Oostra BA, et al. Potential therapeutic interventions for fragile X syndrome. *Trends Mol Med*. 2010;16(11):516–527.
40. Pfeiffer BE, Huber KM. The state of synapses in fragile X syndrome. *Neuroscientist*. 2009;15(5):549–567.
41. Gallagher A, Hallahan B. Fragile X-associated disorders: a clinical overview. *J Neurol*. 2012;259(3):401–413.
42. Penagarikano O, Mulle JG, Warren ST. The pathophysiology of fragile x syndrome. *Annu Rev Genomics Hum Genet*. 2007;8(1):109–129.
43. Willemsen R, Levenga J, Oostra BA. CGG repeat in the FMR1 gene: size matters. *Clin Genet*. 2011;80(3):214–225.
44. Ceman S, O'Donnell WT, Reed M, et al. Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet*. 2003;12(24):3295–3305.
45. Darnell JC, Van Driesche SJ, Zhang C, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell*. 2011;146(2):247–261.
46. Brown V, Jin P, Ceman S, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell*. 2001;107(4):477–487.
47. Muddashetty RS, Kelić S, Gross C, et al. Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. *J Neurosci*. 2007;27(20):5338–5348.
48. Zalfa F, Giorgi M, Primerano B, et al. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell*. 2003;112(3):317–327.
49. Pfeiffer BE, Zang T, Wilkerson JR, et al. Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. *Neuron*. 2010;66(2):191–197.
50. Goebel-Goody SM, Wilson-Wallis ED, Royston S, et al. Genetic manipulation of STEP reverses behavioral abnormalities in a fragile X syndrome mouse model. *Genes Brain Behav*. 2012;11(5):586–600.
51. Schütt J, Falley K, Richter D, et al. Fragile X mental retardation protein regulates the levels of scaffold proteins and glutamate receptors in postsynaptic densities. *J Biol Chem*. 2009;284(38):25479–25487.
52. Paluszkiewicz SM, Martin BS, Huntsman MM. Fragile X syndrome: the GABAergic system and circuit dysfunction. *Dev Neurosci*. 2011;33(5):349–364.
53. Comery TA, Harris JB, Willems PJ, et al. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci U S A*. 1997;94(10):5401–5404.
54. Larson J, Kim D, Patel RC, et al. Olfactory discrimination learning in mice lacking the fragile X mental retardation protein. *Neurobiol Learn Mem*. 2008;90(1):90–102.
55. Koekkoek SK, Yamaguchi K, Milojkovic BA, et al. Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. *Neuron*. 2005;47(3):339–352.
56. Brennan FX, Albeck DS, Paylor R. Fmr1 knockout mice are impaired in a leverpress escape/avoidance task. *Genes Brain Behav*. 2006;5(6):467–471.
57. Mines MA, Yuskaitis CJ, King MK, et al. GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. *PLoS ONE*. 2010;5(3):e9706.
58. Spencer CM, Graham DF, Yuva-Paylor LA, et al. Social behavior in Fmr1 knockout mice carrying a human FMR1 transgene. *Behav Neurosci*. 2008;122(3):710–715.
59. Gibson JR, Bartley AF, Hays SA, et al. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *J Neurophysiol*. 2008;100(5):2615–2626.
60. Qin M, Kang J, Burlin TV, et al. Postadolescent changes in regional cerebral protein synthesis: an in vivo study in the FMR1 null mouse. *J Neurosci*. 2005;25(20):5087–5095.
61. Curia G, Gualtieri F, Bartolomeo R, et al. Resilience to audiogenic seizures is associated with p-ERK1/2 dephosphorylation in the subiculum of Fmr1 knockout mice. *Front Cell Neurosci*. 2013;7:46.
62. Chonchaiya W, Schneider A, Hagerman RJ. Fragile X: a family of disorders. *Adv Pediatr*. 2009;56(1):165–186.
63. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004;27(7):370–377.
64. Gantois I, Vandesompele J, Speleman F, et al. Expression profiling suggests underexpression of the GABA(A) receptor subunit delta in the fragile X knockout mouse model. *Neurobiol Dis*. 2006;21(2):346–357.
65. Gladding CM, Fitzjohn SM, Molnár E. Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms. *Pharmacol Rev*. 2009;61(4):395–412.
66. Collingridge GL, Peineau S, Howland JG, et al. Long-term depression in the CNS. *Nat Rev Neurosci*. 2010;11(7):459–473.
67. Moult PR, Gladding CM, Sanderson TM, et al. Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. *J Neurosci*. 2006;26(9):2544–2554.
68. Xiao MY, Zhou Q, Nicoll RA. Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology*. 2001;41(6):664–671.
69. Waung MW, Pfeiffer BE, Nosyreva ED, et al. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. *Neuron*. 2008;59(1):84–97.
70. Waung MW, Huber KM. Protein translation in synaptic plasticity: mGluR-LTD, Fragile X. *Curr Opin Neurobiol*. 2009;19(3):319–326.
71. Davidkova G, Carroll RC. Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors in hippocampus. *J Neurosci*. 2007;27(48):13273–13278.
72. Hou L, Antion MD, Hu D, et al. Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. *Neuron*. 2006;51(4):441–454.
73. Park S, Park JM, Kim S, et al. Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron*. 2008;59(1):70–83.
74. Zhang Y, Venkitaramani DV, Gladding CM, et al. The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. *J Neurosci*. 2008;28(42):10561–10566.
75. Mao L, Yang L, Tang Q, et al. The scaffold protein Homer1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons. *J Neurosci*. 2005;25(10):2741–2752.
76. Rong R, Ahn JY, Huang H, et al. PI3 kinase enhancer-Homer complex couples mGluRI to PI3 kinase, preventing neuronal apoptosis. *Nat Neurosci*. 2003;6(11):1153–1161.
77. Lyford GL, Yamagata K, Kaufmann WE, et al. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron*. 1995;14(2):433–445.
78. Steward O, Wallace CS, Lyford GL, et al. Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron*. 1998;21(4):741–751.
79. Chowdhury S, Shepherd JD, Okuno H, et al. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron*. 2006;52(3):445–459.
80. Seog DH. Glutamate receptor-interacting protein 1 protein binds to the microtubule-associated protein. *Biosci Biotechnol Biochem*. 2004;68(8):1808–1810.
81. Halpain S, Dehmelt L. The MAP1 family of microtubule-associated proteins. *Genome Biol*. 2006;7(6):224.
82. Maurin JC, Couble ML, Staquet MJ, et al. Microtubule-associated protein 1b, a neuronal marker involved in odontoblast differentiation. *J Endod*. 2009;35(7):992–996.
83. Ronesi JA, Huber KM. Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. *J Neurosci*. 2008;28(2):543–547.
84. Napoli I, Mercaldo V, Boyl PP, et al. The fragile X syndrome protein represses

activity-dependent translation through CYFIP1, a new 4E-BP. *Cell*. 2008;134(6):1042–1054.
85. Wang H, Kim SS, Zhuo M. Roles of fragile X mental retardation protein in dopaminergic stimulation-induced synapse-associated protein synthesis and subsequent alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-4-propionate (AMPA) receptor internalization. *J Biol Chem*. 2010;285(28):21888–21901.
86. Zalfa F, Eleuteri B, Dickson KS, et al. A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. *Nat Neurosci*. 2007;10(5):578–587.
87. Muddashetty RS, Nalavadi VC, Gross C, et al. Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol Cell*. 2011;42(5):673–688.
88. Narayanan U, Nalavadi V, Nakamoto M, et al. FMRP phosphorylation reveals an immediate-early signaling pathway triggered by group I mGluR and mediated by PP2A. *J Neurosci*. 2007;27(52):14349–14357.
89. Dölen G, Osterweil E, Rao BS, et al. Correction of fragile X syndrome in mice. *Neuron*. 2007;56(6):955–962.
90. Krueger DD, Bear MF. Toward fulfilling the promise of molecular medicine in fragile X syndrome. *Annu Rev Med*. 2011;62(1):411–429.
91. Gasparini F, Lingenhöhl K, Stoehr N, et al. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology*. 1999;38(10):1493–1503.
92. Yan QJ, Rammal M, Tranfaglia M, et al. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*. 2005;49(7):1053–1066.
93. de Vrij FM, Levenga J, van der Linde HC, et al. Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. *Neurobiol Dis*. 2008;31(1):127–132.
94. Aschrafi A, Cunningham BA, Edelman GM, et al. The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci U S A*. 2005;102(6):2180–2185.
95. Osterweil EK, Krueger DD, Reinhold K, et al. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *J Neurosci*. 2010;30(46):15616–15627.
96. Michalon A, Sidorov M, Ballard TM, et al. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron*. 2012;74(1):49–56.
97. Heulens I, D'Hulst C, Braat S, et al. Involvement and therapeutic potential of the GABAergic system in the fragile X syndrome. *ScientificWorldJournal*. 2010;10:2198–2206.
98. D'Hulst C, De Geest N, Reeve SP, et al. Decreased expression of the GABAA receptor in fragile X syndrome. *Brain Res*. 2006;1121(1):238–245.
99. Adusei DC, Pacey LK, Chen D, et al. Early developmental alterations in GABAergic protein expression in fragile X knockout mice. *Neuropharmacology*. 2010;59(3):167–171.
100. El Idrissi A, Ding XH, Scalia J, et al. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. *Neurosci Lett*. 2005;377(3):141–146.
101. Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, et al. Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. *J Neurosci*. 2010;30(29):9929–9938.
102. Heulens I, D'Hulst C, Van Dam D, et al. Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. *Behav Brain Res*. 2012;229(1):244–249.
103. Ehrlich I, Humeau Y, Grenier F, et al. Amygdala inhibitory circuits and the control of fear memory. *Neuron*. 2009;62(6):757–771.
104. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6(3):215–229.
105. Semyanov A, Walker MC, Kullmann DM, et al. Tonically active GABA A receptors: modulating gain and maintaining the tone. *Trends Neurosci*. 2004;27(5):262–269.
106. Curia G, Papouin T, Séguéla P, et al. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. *Cereb Cortex*. 2009;19(7):1515–1520.
107. Vigot R, Barbieri S, Bräuner-Osborne H, et al. Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron*. 2006;50(4):589–601.
108. Pacey LK, Doss L, Cifelli C, et al. Genetic deletion of regulator of G-protein signaling 4 (RGS4) rescues a subset of fragile X related phenotypes in the FMR1 knockout mouse. *Mol Cell Neurosci*. 2011;46(3):563–572.
109. Fatemi SH, Folsom TD, Reutiman TJ, et al. Expression of GABA(B) receptors is altered in brains of subjects with autism. *Cerebellum*. 2009;8(1):64–69.
110. Pacey LK, Heximer SP, Hampson DR. Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. *Mol Pharmacol*. 2009;76(1):18–24.
111. Zupan B, Toth M. Inactivation of the maternal fragile X gene results in sensitization of GABA B receptor function in the offspring. *J Pharmacol Exp Ther*. 2008;327(3):820–826.
112. Iossifov I, Ronemus M, Levy D, et al. De novo gene disruptions in children on the autistic spectrum. *Neuron*. 2012;74(2):285–299.
113. Luo Y, Shan G, Guo W, et al. Fragile X mental retardation protein regulates proliferation and differentiation of adult neural stem/progenitor cells. *PLoS Genet*. 2010;6(4):e1000898.
114. Suvrathan A, Hoeffer CA, Wong H, et al. Characterization and reversal of synaptic defects in the amygdala in a mouse model of fragile X syndrome. *Proc Natl Acad Sci U S A*. 2010;107(25):11591–11596.
115. Wilson BM, Cox CL. Absence of metabotropic glutamate receptor-mediated plasticity in the neocortex of fragile X mice. *Proc Natl Acad Sci U S A*. 2007;104(7):2454–2459.
116. Cruz-Martín A, Crespo M, Portera-Cailliau C. Delayed stabilization of dendritic spines in fragile X mice. *J Neurosci*. 2010;30(23):7793–7803.
117. Centonze D, Rossi S, Mercaldo V, et al. Abnormal striatal GABA transmission in the mouse model for the fragile X syndrome. *Biol Psychiatry*. 2008;63(10):963–973.
118. Hoeft F, Hernandez A, Parthasarathy S, et al. Fronto-striatal dysfunction and potential compensatory mechanisms in male adolescents with fragile X syndrome. *Hum Brain Mapp*. 2007;28(6):543–554.
119. Dahlhaus R, El-Husseini A. Altered neuroligin expression is involved in social deficits in a mouse model of the fragile X syndrome. *Behav Brain Res*. 2010;208(1):96–105.
120. Dziembowska M, Pretto DI, Janusz A, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am J Med Genet A*. 2013;161A(8):1897–1903.
121. Bilousova TV, Dansie L, Ngo M, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J Med Genet*. 2009;46(2):94–102.
122. Rotschafer SE, Trujillo MS, Dansie LE, et al. Minocycline treatment reverses ultrasonic vocalization production deficit in a mouse model of Fragile X Syndrome. *Brain Res*. 2012;1439:7–14.
123. Romero-Zerbo Y, Decara J, el Bekay R, et al. Protective effects of melatonin against oxidative stress in Fmr1 knockout mice: a therapeutic research model for the fragile X syndrome. *J Pineal Res*. 2009;46(2):224–234.
124. de Diego-Otero Y, Romero-Zerbo Y, el Bekay R, et al. Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology*. 2009;34(4):1011–1026.
125. Hagerman R, Lauterborn J, Au J, et al. Fragile X syndrome and targeted treatment trials. *Results Probl Cell Differ*. 2012;54:297–335.
126. Wei H, Dobkin C, Sheikh AM, et al. The therapeutic effect of memantine through the stimulation of synapse formation and dendritic spine maturation in autism and fragile X syndrome. *PLoS ONE*. 2012;7(5):e36981.
127. Hagerman RJ, Berry-Kravis E, Kaufmann WE, et al. Advances in the treatment of fragile X syndrome. *Pediatrics*. 2009;123(1):378–390.
128. Rueda JR, Ballesteros J, Tejada MI. Systematic review of pharmacological treatments in fragile X syndrome. *BMC Neurol*. 2009;9(1):53.
129. Hagerman RJ, Murphy MA, Wittenberger MD. A controlled trial of stimulant medication in children with the fragile X syndrome. *Am J Med Genet*. 1988;30(1–2):377–392.
130. Interim guidance about avian influenza A for US citizens living abroad. Centers for Disease Control and Prevention Web site. http://wwwnc.cdc.gov/travel. Updated November 18, 2005. Accessed August 10, 2007.
131. Jacquemont S, Curie A, des Portes V, et al. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci Transl Med*. 2011;3(64):64ra1.
132. Berry-Kravis E, Hessl D, Coffey S, et al. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet*. 2009;46(4):266–271.
133. Berry-Kravis EM, Hessl D, Rathmell B, et al. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Sci Transl Med*. 2012;4(152):152ra127.
134. Erickson CA, Mullett JE, McDougle CJ. Brief report: acamprosate in fragile X syndrome. *J Autism Dev Disord*. 2010;40(11):1412–1416.
135. Wink L, Erickson CA, Early M, et al. A pilot naturalistic trial of acamprosate in idiopathic and fragile X-associated autism spectrum disorders. International Meeting for Autism Research; May 13, 2011; Manchester, United Kingdom.
136. Utari A, Chonchaiya W, Rivera SM, et al. Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome

measures. *Am J Intellect Dev Disabil*. 2010;115(5):433–443.
137. Paribello C, Tao L, Folino A, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurol*. 2010;10(1):91.
138. Leigh MJ, Nguyen DV, Mu Y, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. *J Dev Behav Pediatr*. 2013;34(3):147–155.
139. Berry-Kravis E, Sumis A, Hervey C, et al. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. *J Dev Behav Pediatr*. 2008;29(4):293–302.
140. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J Clin Sleep Med*. 2009;5(2):145–150.
141. Kesler SR, Lightbody AA, Reiss AL. Cholinergic dysfunction in fragile X syndrome and potential intervention: a preliminary 1H MRS study. *Am J Med Genet A*. 2009;149A(3):403–407.
142. Winarni TI, Chonchaiya W, Adams E, et al. Sertraline may improve language developmental trajectory in young children with fragile X syndrome: a retrospective chart review. *Autism Res Treat*. 2012;2012:104317.
143. Erickson CA, Mullett JE, McDougle CJ. Open-label memantine in fragile X syndrome. *J Autism Dev Disord*. 2009;39(12):1629–1635.
144. Erickson CA, Weng N, Weiler IJ, et al. Open-label riluzole in fragile X syndrome. *Brain Res*. 2011;1380:264–270.
145. Berry-Kravis E, Krause SE, Block SS, et al. Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: a controlled trial. *J Child Adolesc Psychopharmacol*. 2006;16(5):525–540.
146. Henderson C, Wijetunge L, Kinoshita MN, et al. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABA B receptors with arbaclofen. *Sci Transl Med*. 2012;4(152):152ra128.
147. Kerrigan JF, Shields WD, Nelson TY, et al. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. *Epilepsy Res*. 2000;42(2–3):133–139.
148. Laxer K, Blum D, Abou-Khalil BW, et al; Ganaxolone Presurgical Study Group. Assessment of ganaxolone's anticonvulsant activity using a randomized, double-blind, presurgical trial design. *Epilepsia*. 2000;41(9):1187–1194.
149. Berry-Kravis E, Sumis A, Hervey C, et al. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. The 11th International Fragile X Conference; July 23–27, 2008; St Louis, Missouri.
150. Hall SS, Lightbody AA, McCarthy BE, et al. Effects of intranasal oxytocin on social anxiety in males with fragile X syndrome. *Psychoneuroendocrinology*. 2012;37(4):509–518.
151. Dawson G, Rogers S, Munson J, et al. Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. *Pediatrics*. 2010;125(1):e17–e23.
152. Restivo L, Ferrari F, Passino E, et al. Enriched environment promotes behavioral and morphological recovery in a mouse model for the fragile X syndrome. *Proc Natl Acad Sci U S A*. 2005;102(32):11557–11562.
153. Winarni TI, Schneider A, Borodyanskara M, et al. Early intervention combined with targeted treatment promotes cognitive and behavioral improvements in young children with fragile X syndrome. *Case Rep Genet*. 2012;2012:280813 .
154. Schneider A, Leigh MJ, Adams P, et al. Electrocortical changes associated with minocycline treatment in fragile X syndrome. *J Psychopharmacol*. 2013;27(10):956–963.
155. Abrams L, Cronister A, Brown WT, et al. Newborn, carrier, and early childhood screening recommendations for fragile X. *Pediatrics*. 2012;130(6):1126–1135.