Molecular medicine of fragile X syndrome: based on known molecular mechanisms

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Background: **Extensive research on fragile X mental retardation gene knockout mice and mutant Drosophila models has largely expanded our knowledge on mechanism-based treatment of fragile X syndrome (FXS). In light of these findings, several clinical trials are now underway for therapeutic translation to humans.**

Data sources: **Electronic literature searches were conducted using the PubMed database and ClinicalTrials.gov. The search terms included "fragile X syndrome", "FXS and medication", "FXS and therapeutics" and "FXS and treatment". Based on the publications identified in this search, we reviewed the neuroanatomical abnormalities in FXS patients and the potential pathogenic mechanisms to monitor the progress of FXS research, from basic studies to clinical trials.**

Results: **The pathological mechanisms of FXS were categorized on the basis of neuroanatomy, synaptic structure, synaptic transmission and fragile X mental retardation protein (FMRP) loss of function. The neuroanatomical abnormalities in FXS were described to motivate extensive research into the region-specific pathologies in the brain responsible for FXS behavioural manifestations. Mechanism-directed molecular medicines were classified according to their target pathological mechanisms, and the most recent progress in clinical trials was discussed.**

Conclusions: **Current mechanism-based studies and clinical trials have greatly contributed to the development of FXS pharmacological therapeutics. Research examining the extent to which these treatments provided a rescue effect or FMRP compensation for the developmental impairments in FXS patients may help to improve the efficacy of treatments.**

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Key words: **clinical trial; fragile X syndrome; mechanism-based; treatment**

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Introduction

Fragile X syndrome (FXS) is the most frequently encountered form of inherited intellectual disability and the most common monogenic cause of autism, with an estimated prevalence of 1 in 7143 males and 1 in 11 111 females.[1,2] The vast majority of cases are caused by a trinucleotide CGG expansion in the 5′-untranslated region of the fragile X mental retardation gene (*FMR1*). Normal alleles contain 6-44 CGG repeats and intermediate alleles contain 45-54 CGG repeats, but premutation alleles contain 55-200 unmethylated CGG repeats. During either oogenesis or early postzygotic events, premutations may undergo further size expansions to methylated full mutations with more than 200 CGG repeats. Hypermethylation and epigenetic silencing of *FMR1* result in the loss of its protein product, fragile X mental retardation protein (FMRP), which causes FXS.[3] The affected individuals may suffer from a cascade of developmental impairments in cognitive functioning and manifest specific behavioural and emotional problems, of which attention dysfunction, hyperactivity, social anxiety, aggression and self-injury are typically the greatest concerns reported by parents and professionals.[4,5] In females with a full mutation, the profile is similar in quality to that of males, but with less severe impairments due to the second, normal functioning X chromosome.[6]

To date, there are no particularly efficient interventions for FXS, though symptom-based medications have been used for maximal functioning of FXS patients. The symptoms most frequently targeted for treatment in males include anxiety (42%), attention (37%),

hyperactivity (27%) and anger or aggression (24%); those less commonly targeted include mood swings (18%), sleep (11%), self-injury (11%), seizures (8%) and depression (8%).[7] A number of medications have been used to manage these symptoms, including stimulants, selective serotonin reuptake inhibitors (SSRIs), antipsychotics and alpha-2 agonists.[8] Several case studies reported a high response rate of symptom-based pharmacological treatments for FXS patients; however, the interpretation of such results was limited by the use of small non-representative study populations and undetermined side effects.[9-12] To address these issues, the efficacy and safety of two stimulants (methylphenidate and dextroamphetamine), two SSRIs (fluoxetine and sertraline), lithium and aripiprazole have been evaluated in pilot trials.[13-18] At present, FXS patients have limited treatment options and there are no Food and Drug Administration-approved medications. Moreover, the available options do not effectively address the core impairments in FXS, but only help the secondary symptoms.[19]

Recent research in *Fmr1* knockout (KO) mice and *dFmr1* mutant *Drosophila* models has elucidated the basic neurobiology of FXS, unravelled disease-oriented pharmacological approaches and revealed the challenges of translating mechanism-targeted therapeutics from animal models to human trials.[20] Translation of FXS-targeted treatments from animal models to humans was previously summarized by Berry-Kravis et al[21] and Wijetunge et al.[22] In the present review, we discuss the neuroanatomical changes in FXS and the corresponding mechanism-based treatments from the perspectives of neuroanatomy, synaptic structure, signal transmission, and FMRP loss of function. We also present the most recent progress in translational molecular medicine and drug effects using corresponding outcome measures.

Neuroanatomy of FXS

Over the past decade, structural and functional magnetic resonance imaging (MRI) studies in FXS patients have revealed abnormalities in specific brain structures. Much effort has been made to integrate the findings of these studies, concerning the inconsistencies associated with mixed gender populations, combined sampling, differential morphometric methods and the analytical strategies used to measure specific brain regions. One of the most striking findings is a significantly enlarged caudate nucleus (CN). The CN is part of the basal ganglia and plays a key role in the frontal-subcortical circuits that are important for maintaining and shifting attention, executive function, emotional

Table. Mechanism-based molecular medicines in FXS

Target	Agent	Phenotypes reversed in *Fmr1* KO mouse	Translational progress in human trials
mGluR system	Fenobam	Protrusion morphology, associative motor learning and avoidance behavior	PPI improved, hyperactivity and anxiety reduced (open-label)
	AFQ056	PPI and spine shape, sociability behavior	Behavior improved in 7 fully methylated patients (phase IIb), no overall improvement (phase IIb/III), extension trials discontinued
	RO4917523		Phase II trial completed
	Lithium	Learning deficit, open-field hyperactivity, passive avoidance and social behaviors, partially normalize anxiety level and spine morphology, macroorchidism, audiogenic seizures, cognitive impairments	Global behavior improved, some adaptive functioning and language use improved, ERK biomarker normalized (open-label)
GABA system	Ganaxolone	Audiogenic seizures	Phase II trial recruiting
	STX209	Audiogenic seizures, open-field hyperactivity, spine density	Social and language function improved in the most severely socially impaired subgroup (phase II), no overall improvement (phase III), extension trials discontinued
AMPAR system and others	CX516		No overall improvement (phase II)
	Riluzole		No overall improvement, ERK biomarker normalized (open-label)
	Acamprosate		Language and socialization improved in 3 patients, social behavior improved and inattention/hyperactivity reduced (open-label)
Polyribosome stalling	Minocycline	Dendritic spine morphology and anxiety, vocalization deficits, locomotor activity, partially attenuates audiogenic seizures	Global behavior improved (phase II)
FMR1 reactivation	LAC		Social behavior improved and hyperactivity reduced (phase II)

FXS: fragile X syndrome; *FMR1*: fragile X mental retardation gene; PPI: prepulse inhibition; ERK: extracellular signal-regulated kinase; GABA: gamma-aminobutyric acid; LAC: acetyl-L-carnitine; AMPAR: aminomethyl phosphonic acid receptor; KO: knockout; mGluR: metabotropic glutamate receptor.

liability, motor programming and oculomotor functions, all of which are disrupted in FXS.[17,23-28] Correlations between focal CN topography and frontal lobe-associated cognitive and behavioural deficits in FXS patients indicate that an enlarged CN is associated with the abnormal orbitofrontal-caudate and dorsolateral-caudate circuitry in FXS.[29] Neurometabolite levels measured by proton magnetic resonance spectroscopy have further revealed reduced ratios of choline/creatine and glutamate+glutamine/creatine in the CN of FXS patients.[30]

Another consistent finding in both FXS males and females is a smaller posterior cerebellar vermis, a structure that is important for processing sensory stimuli and performing sensorimotor integration.[25,26,31] Correlations between the findings from MRI and cognitive/behavioural tests have revealed that the size of the posterior cerebellar vermis as a significant predictor of an individual's performance on most of the cognitive measures examined is negatively correlated with stereotypic/restricted behaviour measures, communication dysfunction and autistic items.[31,32] Neurometabolite profiling of FXS pediatric patients fails to reveal statistically significant differences in the ratios of choline/creatine, N-acetyl aspartate/creatine and choline/N-acetyl aspartate in cerebellar vermian voxels.[33]

FXS patients have shown reduced volumes in the insula, a sensory integration region increasingly associated with anxiety. This deficiency could account for the prominent hyperarousal and gaze aversion symptoms in FXS patients.[34] FXS children and adolescents have demonstrated increased ventricular cerebral spinal fluid volumes and reductions in the size of the amygdala and superior temporal gyrus.[24,25,28] However, earlier findings in FXS patients regarding the size of the hippocampus, a key structure in learning and memory that has high *FMR1* mRNA levels during fetal development, remain inconclusive.[17]

Recent longitudinal neuroimagings have elucidated early neurodevelopmental patterns in young FXS children. Hoeft et al[26] observed an enlarged voxel-wise grey matter volume (GMV) in the caudate, thalamus and fusiform gyri, as well as a reduced GMV in the cerebellar vermis in 1- to 3-year-old FXS boys. The initial GMV of the orbital gyri, basal forebrain and thalamus in FXS patients was similar to that of the controls; however, the patients showed the GMV increases on an altered growth trajectory, suggesting disrupted postnatal synaptic pruning. Larger white matter volume in the striatal–prefrontal regions has also been reported. Hazlett et al[28] monitored the structural brain volumes of FXS boys at the age of 2-3 years and again at the age of 4-5 years. In contrast to children with idiopathic autism with generalized cortical lobe enlargement, FXS children showed specific enlargement in the temporal lobe white matter, cerebellar grey matter and CN, but a significantly smaller amygdala, which might help to differentiate between FXS and idiopathic autism.

Current understanding of the molecular and cellular mechanisms in FXS is mostly derived from studies on the hippocampus and cortex of *Fmr1* KO mice. Amygdala dysfunction has also been tentatively linked with the strong emotional symptoms of FXS.[35,36] However, how these anatomical abnormalities could cause synaptic dysfunction in FXS patients remains largely unexplored. Neurometabolite profiling in FXS patients revealed aberrant basal ganglia metabolism.[30] Identification of specific neurometabolites within hippocampus-centric and multiple brain sites may help to provide critical metabolic signatures and biomarkers for determining the efficacy of disease-specific pharmacological treatments. It is also important to find synaptic dysfunction within these aberrant brain areas, which may unravel site-specific pathogenic mechanisms and their associated behavioural manifestations.

Targeted treatment of FXS

The translation of mechanism-based therapeutics from animal models to clinical populations is organized by the impaired transmission system, including the metabotropic glutamate receptor (mGluR), gamma-aminobutyric acid (GABA) and aminomethyl phosphonic acid receptor (AMPAR) as well as their presynaptic systems, and the aspect of FMRP loss-of-function to outline the progress from basic research studies to clinical trials (Table).

mGluR system in FXS

An early neuroanatomical study of the *Fmr1* KO mouse brain using *in vivo* MRI found no evidence for size alterations in the brain regions that are typically abnormal in FXS patients, including the cerebellar vermis, subcortical grey (e.g., caudate, lenticular and thalamic nuclei), hippocampus and total brain.[37] Further characterization of the anatomical changes in *Fmr1* KO mice revealed significant decreases of volume in two deep cerebellar nuclei, the nucleus interpositus and fastigial nucleus, whereas how neuronal loss in these regions might contribute to the pathogenesis of FXS remains to be investigated.[38] Although murine MRI studies showed no significant differences in *Fmr1* KO mice, a morphology pattern of long, thin, tortuous dendritic spines was observed in the Golgi-impregnated cerebral cortex of *Fmr1* KO mice similar

to that observed in FXS patients, as well as a reduced seizure threshold.[39-44] mGluR long-term depression (LTD) is exaggerated in the hippocampus of *Fmr1* KO mice, and FMRP is normally synthesized after the stimulation of group 1 mGluRs. Based on these findings, Bear et al[45,46] first proposed the current prevailing mGluR theory. Normally, mGluR activation stimulates synthesis of proteins involved in LTD stabilization and FMRP, which inhibits further synthesis and halts LTD. Consistent with this mechanism of mGluR overactivity in FXS, 2-methyl-6-(phenylethynyl)-pyridine and other mGluR5 negative modulators (CTEP, AFQ056) could reverse learning deficits and multiple phenotypes in the *Fmr1* KO mouse that included audiogenic seizures, epileptiform discharges, open field hyperactivity, spine dysmorphology and neural activity patterns.[47-52] Furthermore, a 50% reduction in mGluR5 expression in the *Fmr1* KO mice, which specifically rescued the immature spine phenotype, elevated synaptic protein synthesis and fewer audiogenic seizures, strongly supports this theory and identifies potential targets for FXS pharmacological therapeutics.[53]

However, trials for mGluR5 antagonists in FXS adults have shown limited effects. Berry-Kravis et al[54] conducted a pilot open-label, single-dose trial with fenobam in 12 adult FXS males and females. No significant adverse reactions to fenobam were observed, and the patients showed a positive behavioural response that included improvements in communication, eye contact and prepulse inhibition deficits. The next mGluR5 antagonist, AFQ056, was studied in a European phase II trial (randomised, double-blind, crossover design; Novartis) including 30 FXS male patients aged 18-35 years. Seven patients with full *FMR1* promoter methylation significantly responded to AFQ056 treatment according to their primary outcome measures, the Aberrant Behaviour Checklist (ABC) and the Clinical Global Impression (CGI) scale, in addition to most of the secondary outcome measures compared with placebo.[55] More recently, Novartis has discontinued the development of mavoglurant (AFQ056) in FXS and closed the current open-label extension trials, based on the phase IIb/III studies with mavoglurant (AFQ056), in which FXS adolescents and adults did not meet the primary and secondary endpoints of showing improvement in abnormal behaviours compared with placebo.[56] Another mGluR5 antagonist is currently being assessed in multicentre clinical trials (RO4917523, Roche Pharmaceuticals). A phase II randomized, double-blind, placebo-controlled, parallel-arm study of RO4917523 in fragile X adults was recently completed, but the outcome measure results are pending. Studies on RO4917523 in pediatric, adolescent and adult patients using a longer treatment period (12 weeks) are currently in progress. We anticipated that these prospective studies may introduce fresh and inspiring outcomes for FXS-targeted treatment.

Activation of signalling pathways under the mGluR system, such as the extracellular signal-regulated kinase (ERK)-mitogen-activated protein kinase and phosphatidyl inositol 3-kinase-mammalian target of rapamycin (mTOR) pathways, is dysregulated in *Fmr1* KO mice and has been targeted for specific treatment.[57,58] Lithium, associated with normalization of excessive ERK and glycogen synthase kinase-3β in the *Fmr1* KO mouse, improved both immediate and short-term memory deficits in dfxr mutant flies and alleviated impaired cognition, behavioural and morphological abnormalities in the *Fmr1* KO mouse.[59-62] A pilot open-label trial in 15 FXS patients aged 6-23 years showed significant behavioural improvement on measures including the total ABC-C score, ABC-Social Withdrawal subscale and CGI scale.[18]

The GABA system in relation to FXS

A lack of FMRP in animal models was found to be associated with deficient GABA signalling.[63,64] GABA-A receptor expression is significantly downregulated in the cortex of *Fmr1* KO mice, and mRNAs encoding GABA-A receptor subunits are direct targets of FMRP.[63,64] GABA-A agonists ganaxolone and taurine, which act to directly compensate for the GABA-A subunit deficiencies, reduce audiogenic seizures and improve learning in a passive avoidance test, respectively.[65,66] The Medical Investigation of Neurodevelopmental Disorders Institute (UC Davis, CA, USA) is now actively recruiting participants for a phase II, double-blind, randomized, placebo-controlled, crossover study to investigate ganaxolone treatment in FXS children. Improvements in anxiety and attention are anticipated.

GABA-B receptor agonists inhibit presynaptic glutamate release, postsynaptic transmission and intracellular signalling cascades downstream of mGluR5 and thus may indirectly serve as an inhibitor of the mGluR5 pathway.[67] The GABA-B agonist STX209 (arbaclofen, R-baclofen) rescued the audiogenic phenotype, corrected the increased dendritic spine density and elevated basal protein synthesis and AMPAR internalization in the *Fmr1* KO mouse.[68] In a randomized, double-blind, placebo-controlled crossover phase II trial, Berry-Kravis et al[69] found that STX209 was well tolerated with mild side effects. However, no improvement was seen on the ABC-Irritability subscale, the study's primary endpoint, whereas the post hoc analysis with the ABC-Social Avoidance scale showed signs of improvement in social function, particularly in the most severely socially impaired subgroup. Unfortunately, in a recent phase III trial, STX209 treatment missed its main goal of reducing social withdrawal in FXS patients, which lead to

the termination of planned extension studies of STX209.[70]

The AMPAR system and other factors related to FXS

In addition to the mGluR5 and GABA systems, the effects of other neurotransmission systems including the AMPAR, the N-methyl-D-aspartate receptor (NMDAR), and brain-derived neurotrophic factor (BDNF) have also been explored in FXS with available modulators.

Dysregulated mGluR-dependent translation of AMPAR subunits (GluR1/2) was discovered in the synaptoneurosomes of *Fmr1* KO mice.[71] It was also found that FMRP reduction leads to excessive mGluR5-dependent internalisation of the AMPAR subunit GluR1 in the dendrites of cultured rat hippocampal neurons.[72] A 4-week randomized, double-blind, placebo-controlled phase II clinical trial with CX516 treatment, a direct AMPAR-positive modulator that can increase long-term potentiation and BDNF levels,[73,74] found no significant improvement in CX516-treated subjects compared with those treated with placebo, which is likely because CX516 is a very weak ampakine and provides only weak BDNF induction.

Riluzole, a sodium channel blocker and glutamate uptake activator, was hypothesized to attenuate glutamate-induced excitotoxicity and potentiate postsynaptic GABA-A receptor activity. In combination with early positive reports on targeting treatment-resistant depression and obsessive-compulsive disorder, a 6-week open-label prospective pilot study on riluzole (100 mg/day) was conducted in six FXS adults. Despite uniform correction of peripheral ERK activation, riluzole was not associated with significant clinical improvement.[75]

Acamprosate, a novel agent with potential pleiotropic effects and acting as an NMDA glutamate receptor antagonist, potential mGluR5 antagonist and GABA-A agonist in animal studies, was evaluated in an initial pilot open-label study in three patients with FXS and a comorbid diagnosis of autistic disorder for over 21.3 weeks. This trial showed marked improvement in communication in all patients as rated using the CGIs-Improvement scale.[76] A subsequent open-label 10-week trial with acamprosate in 12 FXS patients aged between 6 and 17 years indicated an association with significant improvement in social behaviour and a reduction in inattention/hyperactivity with general safety and tolerance. In addition, an increase in BDNF that occurred with treatment may be a useful pharmacodynamic marker in future acamprosate studies.[77]

Presynaptic deficits in FXS

The aforementioned therapeutic targets for FXS have largely focused on postsynaptic deficits. The potential roles of FMRP at the presynaptic apparatus have been gradually examined.[78] FMRP is expressed in presynaptic terminals and axons of fragile X granules (FXG)-enriched brain circuits and regulates FXG number and developmental profile.[79] High throughput sequencing of RNA isolated by crosslinking immunoprecipitation indicated that FMRP interacts directly with mRNA encoding nearly one-third of the presynaptic proteome.[80] Enhanced responses to high-frequency stimulation in *Fmr1* KO mice are associated with an exaggerated calcium influx in presynaptic neurons, enhanced vesicle recycling and enlarged readily releasable and reserved vesicle pools.[81] Loss of FMRP also leads to excessive action potential broadening during repetitive activity, enhanced presynaptic calcium influx and elevated neurotransmitter release in cornu ammonis fields CA3 pyramidal neurons.[82] It was recently reported that FMRP controls synaptic vesicle exocytosis by modulating N-type calcium channel density.[83] As a potential contributor to neurological impairment in FXS, presynaptic dysfunction deserves extensive research to identify potential intervention targets.

FMRP and polyribosome stalling in FXS

A body of literature has shown that FMRP is a neuronal polyribosome-associated RNA-binding protein that binds to approximately 4% of all brain mRNAs and subsequently modulates translation of a set of synaptic plasticity-related proteins by stalling ribosomal translocation on target mRNAs.[80,84] Recently, Darnell et al[85] have proposed an interesting point that a secondary consequence of dysregulation of the primary mRNA targets of FMRP could account for the deficits observed in FXS patients and animal models. Given that FMRP normally inhibits the expression of mGluR5 receptors, NMDARs and components of downstream ERK and mTORC1 pathways, as well as several potentially limiting and elongation factors, excess translation of these proteins in the chronic absence of FMRP could lead to secondary increases in global protein synthesis. It has also been proposed that small molecules to stall ribosomes in the absence of FMRP have the potential to be translated into new therapeutic avenues for the treatment of FXS.[85]

In consistency, genetic reduction of p70 ribosomal protein S6 kinase 1, targeting direct translation machinery, has been reported to restore proper translational control and protein synthesis, normal mGluR-LTD and dendritic morphology in FXS model mice, as well as weight gain, macro-orchidism and multiple autism spectrum disorder-like behavioural phenotypes, including social interaction deficits, impaired novel objection recognition and behavioural

inflexibility.[86] Double KO mice of *Fmr1* and cytoplasmic polyadenylation element binding protein 1 gene, which were predicted to bind many of the same mRNAs and mediate a translational homeostasis, also ameliorated the biochemical, morphological, electrophysiological and behavioural phenotypes associated with FXS.[87] In addition, application of minocycline, a second-generation tetracycline analogue that inhibits matrix metalloproteinase-9 and stalls mammalian ribosomal translocation, also reverses synaptic abnormalities and aberrant behaviours in *Fmr1* KO mice and *Fmr1* null mutant flies.[88-90] A randomized double-blind, placebo-controlled, crossover trial of minocycline in 55 FXS children and adolescents (3.5-16 years old) has been conducted for 3 months. In spite of the placebo effect, significant improvements have been shown in primary CGI scores, anxiety and mood-related behaviours.[91]

FMR1 reactivation

Attempts have been made to reactivate *FMR1* transcription by removing the transcriptional blockade induced by excess promoter methylation. Demethylation agents such as 5-azadeoxycytidine can increase *FMR1* mRNA levels and protein in the lymphoblasts of FXS patients;[92] however, the high toxicity of its long-term use and requirements of incorporating it into dividing cells have limited its application. Histone hyper-acetylating drug that can reduce *in vitro* expression of the fragile site of FXS without affecting DNA methylation, the acetyl-L-carnitine (LAC), has been explored in a randomized, double-blind, placebo-controlled, parallel and multicenter study.[93] LAC appears to be well tolerated in the overall population and could reduce hyperactivity in FXS boys with attention deficit hyperactivity disorder (ADHD), whereas another multi-site 16-week pilot randomized controlled trial found that LAC was ineffective in ADHD children (n=122).[94] The efficacy of LAC on ADHD needs to be further evaluated.

Conclusions

FXS is the most common inherited form of intellectual disability and the most common known cause of autism. The extraordinary research progress with FXS animal models has provided explicit insights into potential mechanism-targeted medications. Extensive effort has been made to standardize a set of disease-specific outcome measures for comprehensive evaluation of drug efficacy. Current clinical trials have documented several promising agents of positive behavioural effects.

It should be emphasized that FMRP is expressed ubiquitously in different types of neurons beginning in the fetal developmental period. The extent to which that late-stage pharmacological therapeutics could rescue the established deficits at specific neuroanatomical sites in FXS patients requires further investigation. Although genetic and pharmacological manipulations of *Fmr1* KO mice could partly reverse synaptic abnormalities, evaluation of recovery of normal synaptic functioning has been limited by the lack of consistent medical biomarkers. FMRP targets approximately 30% of the postsynaptic density and presynaptic proteome, and regulates protein translation through multiple roles in polyribosome stalling, mRNA transport and microRNA pathway. Insufficient expression of FMRP thus leads to a broad array of effects on synaptic transmission, structure and function.

Therefore, the ideal FXS treatment should meet two conditions. First, it should compensate for the temporary functioning of FMRP or at least the major function responsible for the targeted symptoms. Second, it should restore the developmental deficits caused by long-term chronic loss of FMRP. To facilitate this goal, a better understanding and incorporation of FXS pathology, from the developmental neuroanatomical changes, immature dendritic spines, altered pre- and postsynaptic transmissions to secondary consequence of dysregulated FMRP targets, must pave the way to a comprehensive and efficacious treatment strategy for the cognitive and behavioural problems associated with FXS.

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References

1 Hagerman R, Hoem G, Hagerman P. Fragile X and autism: intertwined at the molecular level leading to targeted treatments. Mol Autism 2010;1:12.
2 Hunter J, Rivero-Arias O, Angelov A, Kim E, Fotheringham I, Leal J. Epidemiology of fragile X syndrome: a systematic review and meta-analysis. Am J Med Genet A 2014;164A:1648-1658.
3 Wang T, Bray SM, Warren ST. New perspectives on the biology of fragile X syndrome. Curr Opin Genet Dev 2012;22:256-263.
4 Reiss AL, Hall SS. Fragile X syndrome: assessment and treatment implications. Child Adolesc Psychiatr Clin N Am 2007;16:663-675.
5 Hatton DD, Hooper SR, Bailey DB, Skinner ML, Sullivan KM, Wheeler A. Problem behavior in boys with fragile X syndrome.

Am J Med Genet 2002;108:105-116.
6 Finucane B, Abrams L, Cronister A, Archibald AD, Bennett RL, McConkie-Rosell A. Genetic counseling and testing for *FMR1* gene mutations: practice guidelines of the national society of genetic counselors. J Genet Couns 2012;21:752-760.
7 Bailey DB Jr, Raspa M, Bishop E, Olmsted M, Mallya UG, Berry-Kravis E. Medication utilization for targeted symptoms in children and adults with fragile X syndrome: US survey. J Dev Behav Pediatr 2012;33:62-69.
8 Berry-Kravis E, Potanos K. Psychopharmacology in fragile X syndrome-present and future. Ment Retard Dev Disabil Res Rev 2004;10:42-48.
9 Brown WT, Jenkins EC, Friedman E, Brooks J, Cohen IL, Duncan C, et al. Folic acid therapy in the fragile X syndrome. Am J Med Genet 1984;17:289-297.
10 Hilton DK, Martin CA, Heffron WM, Hall BD, Johnson GL. Imipramine treatment of ADHD in a fragile X child. J Am Acad Child Adolesc Psychiatry 1991;30:831-834.
11 Cohen IL, Tsiouris JA, Pfadt A. Effects of long-acting propranolol on agonistic and stereotyped behaviors in a man with pervasive developmental disorder and fragile X syndrome: a double-blind, placebo-controlled study. J Clin Psychopharmacol 1991;11:398-399.
12 Strom CM, Brusca RM, Pizzi WJ. Double-blind, placebo-controlled crossover study of folinic acid (Leucovorin) for the treatment of fragile X syndrome. Am J Med Genet 1992;44:676-682.
13 Hagerman RJ, Murphy MA, Wittenberger MD. A controlled trial of stimulant medication in children with the fragile X syndrome. Am J Med Genet 1988;30:377-392.
14 Erickson CA, Stigler KA, Wink LK, Mullett JE, Kohn A, Posey DJ, et al. A prospective open-label study of aripiprazole in fragile X syndrome. Psychopharmacology (Berl) 2011;216:85-90.
15 Hagerman RJ, Hills J, Scharfenaker S, Lewis H. Fragile X syndrome and selective mutism. Am J Med Genet 1999;83:313-317.
16 Indah Winarni T, Chonchaiya W, Adams E, Au J, Mu Y, Rivera SM, 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.
17 Hagerman RJ, Hagerman PJ. Fragile X syndrome: diagnosis, treatment, and research. Baltimore: Johns Hopkins University Press, 2002.
18 Berry-Kravis E, Sumis A, Hervey C, Nelson M, Porges SW, Weng N, et al. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. J Dev Behav Pediatr 2008;29:293-302.
19 National Fragile X Foundation, 2014. www.fragilex.org/2014/research/opportunities-for-families/alcobra-ltd-announces-a-new-clinical-trial-enrolling-subjects-with-fragile-x-syndrome-in-2014 (accessed July 11, 2014).
20 Jacquemont S, Berry-Kravis E, Hagerman R, von Raison F, Gasparini F, Apostol G, et al. The challenges of clinical trials in fragile X syndrome. Psychopharmacology (Berl) 2014;231:1237-1250.
21 Berry-Kravis E, Knox A, Hervey C. Targeted treatments for fragile X syndrome. J Neurodev Disord 2011;3:193-210.
22 Wijetunge LS, Chattarji S, Wyllie DJ, Kind PC. Fragile X syndrome: from targets to treatments. Neuropharmacology 2013;68:83-96.
23 Reiss AL, Abrams MT, Greenlaw R, Freund L, Denckla MB. Neurodevelopmental effects of the FMR-1 full mutation in humans. Nat Med 1995;1:159-167.
24 Eliez S, Blasey CM, Freund LS, Hastie T, Reiss AL. Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. Brain 2001;124:1610-1618.
25 Gothelf D, Furfaro JA, Hoeft F, Eckert MA, Hall SS, O'Hara R, et al. Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). Ann Neurol 2008;63:40-51.
26 Hoeft F, Carter JC, Lightbody AA, Cody Hazlett H, Piven J, Reiss AL. Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. Proc Natl Acad Sci U S A 2010;107:9335-9339.
27 Hallahan BP, Craig MC, Toal F, Daly EM, Moore CJ, Ambikapathy A, et al. *In vivo* brain anatomy of adult males with Fragile X syndrome: an MRI study. Neuroimage 2011;54:16-24.
28 Hazlett HC, Poe MD, Lightbody AA, Styner M, MacFall JR, Reiss AL, et al. Trajectories of early brain volume development in fragile X syndrome and autism. J Am Acad Child Adolesc Psychiatry 2012;51:921-933.
29 Peng DX, Kelley RG, Quintin EM, Raman M, Thompson PM, Reiss AL. Cognitive and behavioral correlates of caudate subregion shape variation in fragile X syndrome. Hum Brain Mapp 2014;35:2861-2868.
30 Bruno JL, Shelly EW, Quintin EM, Rostami M, Patnaik S, Spielman D, et al. Aberrant basal ganglia metabolism in fragile X syndrome: a magnetic resonance spectroscopy study. J Neurodev Disord 2013;5:20.
31 Mostofsky SH, Mazzocco MM, Aakalu G, Warsofsky IS, Denckla MB, Reiss AL. Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. Neurology 1998;50:121-130.
32 Mazzocco MM, Kates WR, Baumgardner TL, Freund LS, Reiss AL. Autistic behaviors among girls with fragile X syndrome. J Autism Dev Disord 1997;27:415-435.
33 Utine GE, Akpınar B, Arslan U, Kiper PÖ, Volkan-Salancı B, Alanay Y, et al. Neurochemical evaluation of brain function with 1H magnetic resonance spectroscopy in patients with fragile X syndrome. Am J Med Genet A 2014;164A:99-105.
34 Cohen JD, Nichols T, Brignone L, Hall SS, Reiss AL. Insular volume reduction in fragile X syndrome. Int J Dev Neurosci 2011;29:489-494.
35 Suvrathan A, Hoeffer CA, Wong H, Klann E, Chattarji S. 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;107:11591-11596.
36 Suvrathan A, Chattarji S. Fragile X syndrome and the amygdala. Curr Opin Neurobiol 2011;21:509-515.
37 Kooy RF, Reyniers E, Verhoye M, Sijbers J, Bakker CE, Oostra BA, et al. Neuroanatomy of the fragile X knockout mouse brain studied using *in vivo* high resolution magnetic resonance imaging. Eur J Hum Genet 1999;7:526-532.
38 Ellegood J, Pacey LK, Hampson DR, Lerch JP, Henkelman RM. Anatomical phenotyping in a mouse model of fragile X syndrome with magnetic resonance imaging. Neuroimage 2010;53:1023-1029.
39 Hinton VJ, Brown WT, Wisniewski K, Rudelli RD. Analysis of neocortex in three males with the fragile X syndrome. Am J Med Genet 1991;41:289-294.
40 Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet 2001;98:161-167.

41 Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, et al. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc Natl Acad Sci U S A 1997;94:5401-5404.
42 Irwin SA, Idupulapati M, Gilbert ME, Harris JB, Chakravarti AB, Rogers EJ, et al. Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. Am J Med Genet 2002;111:140-146.
43 McKinney BC, Grossman AW, Elisseou NM, Greenough WT. Dendritic spine abnormalities in the occipital cortex of C57BL/6 *Fmr1* knockout mice. Am J Med Genet B Neuropsychiatr Genet 2005;136B:98-102.
44 Chen L, Toth M. Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience 2001;103:1043-1050.
45 Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 2002;99:7746-7750.
46 Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004;27:370-377.
47 Gandhi RM, Kogan CS, Messier C. 2-Methyl-6-(phenylethynyl) pyridine (MPEP) reverses maze learning and PSD-95 deficits in *Fmr1* knock-out mice. Front Cell Neurosci 2014;8:70.
48 Chuang SC, Zhao W, Bauchwitz R, Yan Q, Bianchi R, Wong RK. Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. J Neurosci 2005;25:8048-8055.
49 Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 2005;49:1053-1066.
50 Su T, Fan HX, Jiang T, Sun WW, Den WY, Gao MM, et al. Early continuous inhibition of group 1 mGlu signaling partially rescues dendritic spine abnormalities in the *Fmr1* knockout mouse model for fragile X syndrome. Psychopharmacology (Berl) 2011;215:291-300.
51 Pop AS, Levenga J, de Esch CE, Buijsen RA, Nieuwenhuizen IM, Li T, et al. Rescue of dendritic spine phenotype in *Fmr1* KO mice with the mGluR5 antagonist AFQ056/Mavoglurant. Psychopharmacology (Berl) 2014;231:1227-1235.
52 Michalon A, Bruns A, Risterucci C, Honer M, Ballard TM, Ozmen L, et al. Chronic metabotropic glutamate receptor 5 inhibition corrects local alterations of brain activity and improves cognitive performance in fragile X mice. Biol Psychiatry 2014;75:189-197.
53 Dölen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, et al. Correction of fragile X syndrome in mice. Neuron 2007;56:955-962.
54 Berry-Kravis E, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, et al. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. J Med Genet 2009;46:266-271.
55 Jacquemont S, Curie A, des Portes V, Torrioli MG, Berry-Kravis E, Hagerman RJ, 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;3:64ra1.
56 National Fragile X Foundation, 2014. www.fragilex.org/2014/research/news-reports-and-commentaries/novartis-announces-results-of-mavoglurant-mglur5-afq056-clinical-trials-and-the-conclusion-of-the-long-term-extension-study (accessed July 11, 2014).
57 Kim SH, Markham JA, Weiler IJ, Greenough WT. Aberrant early-phase ERK inactivation impedes neuronal function in fragile X syndrome. Proc Natl Acad Sci U S A 2008;105:4429-4434.
58 Sharma A, Hoeffer CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, et al. Dysregulation of mTOR signaling in fragile X syndrome. J Neurosci 2010;30:694-702.
59 McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron 2005;45:753-764.
60 Yuskaitis CJ, Mines MA, King MK, Sweatt JD, Miller CA, Jope RS. Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. Biochem Pharmacol 2010;79:632-646.
61 King MK, Jope RS. Lithium treatment alleviates impaired cognition in a mouse model of fragile X syndrome. Genes Brain Behav 2013;12:723-731.
62 Chen X, Sun W, Pan Y, Yang Q, Cao K, Zhang J, et al. Lithium ameliorates open-field and elevated plus maze behaviors, and brain phospho-glycogen synthase kinase 3-beta expression in fragile X syndrome model mice. Neurosciences (Riyadh) 2013;18:356-362.
63 D'Hulst C, De Geest N, Reeve SP, Van Dam D, De Deyn PP, Hassan BA, et al. Decreased expression of the GABAA receptor in fragile X syndrome. Brain Res 2006;1121:238-245.
64 Miyashiro KY, Beckel-Mitchener A, Purk TP, Becker KG, Barret T, Liu L, et al. RNA cargoes associating with FMRP reveal deficits in cellular functioning in *Fmr1* null mice. Neuron 2003;37:417-431.
65 Heulens I, D'Hulst C, Van Dam D, De Deyn PP, Kooy RF. Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behav Brain Res 2012;229:244-249.
66 El Idrissi A, Boukarrou L, Dokin C, Brown WT. Taurine improves congestive functions in a mouse model of fragile X syndrome. Adv Exp Med Biol 2009;643:191-198.
67 Isaacson JS, Hille B. GABA(B)-mediated presynaptic inhibition of excitatory transmission and synaptic vesicle dynamics in cultured hippocampal neurons. Neuron 1997;18:143-152.
68 Henderson C, Wijetunge L, Kinoshita MN, Shumway M, Hammond RS, Postma FR, et al. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. Sci Transl Med 2012;4:152ra128.
69 Berry-Kravis EM, Hessl D, Rathmell B, Zarevics P, Cherubini M, Walton-Bowen K, 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;4:152ra127.
70 FRAXA Research Foundation, 2013. http://www.fraxa.org/seaside-stx209-arbaclofen (accessed July 11, 2014).
71 Muddashetty RS, Kelić S, Gross C, Xu M, Bassell GJ. 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;27:5338-5348.
72 Nakamoto M, Nalavadi V, Epstein MP, Narayanan U, Bassell GJ, Warren ST. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. Proc Natl Acad Sci U S A 2007;104:15537-15542.
73. Berry-Kravis E, Krause SE, Block SS, Guter S, Wuu J, Leurgans S, 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;16:525-540.
74 Jourdi H, Hsu YT, Zhou M, Qin Q, Bi X, Baudry M. Positive AMPA receptor modulation rapidly stimulates BDNF release and increases dendritic mRNA translation. J Neurosci 2009;29:8688-8697.
75 Erickson CA, Weng N, Weiler IJ, Greenough WT, Stigler KA, Wink LK, et al. Open-label riluzole in fragile X syndrome. Brain Res 2011;1380:264-270.
76 Erickson CA, Mullett JE, McDougle CJ. Brief report: acamprosate in fragile X syndrome. J Autism Dev Disord 2010;40:1412-1416.
77 Erickson CA, Wink LK, Ray B, Early MC, Stiegelmeyer E, Mathieu-Frasier L, et al. Impact of acamprosate on behavior and brain-derived neurotrophic factor: an open-label study in youth with fragile X syndrome. Psychopharmacology (Berl) 2013;228:75-84.
78 Wang T, Bray SM, Warren ST. New perspectives on the biology of fragile X syndrome. Curr Opin Genet Dev 2012;22:256-263.
79 Christie SB, Akins MR, Schwob JE, Fallon JR. The FXG: a presynaptic fragile X granule expressed in a subset of developing brain circuits. J Neurosci 2009;29:1514-1524.
80 Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 2011;146:247-261.
81 Deng PY, Sojka D, Klyachko VA. Abnormal presynaptic short-term plasticity and information processing in a mouse model of fragile X syndrome. J Neurosci 2011;31:10971-10982.
82 Deng PY, Rotman Z, Blundon JA, Cho Y, Cui J, Cavalli V, et al. FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. Neuron 2013;77:696-711.
83 Ferron L, Nieto-Rostro M, Cassidy JS, Dolphin AC. Fragile X mental retardation protein controls synaptic vesicle exocytosis by modulating N-type calcium channel density. Nat Commun 2014;5:3628.
84 Khandjian EW, Corbin F, Woerly S, Rousseau F. The fragile X mental retardation protein is associated with ribosomes. Nat Genet 1996;12:91-93.
85 Darnell JC, Klann E. The translation of translational control by FMRP: therapeutic targets for FXS. Nat Neurosci 2013;16:1530-1536.
86 Bhattacharya A, Kaphzan H, Alvarez-Dieppa AC, Murphy JP, Pierre P, Klann E. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. Neuron 2012;76:325-337.
87 Udagawa T, Farny NG, Jakovcevski M, Kaphzan H, Alarcon JM, Anilkumar S, et al. Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology. Nat Med 2013;19:1473-1477.
88 Dziembowska M, Pretto DI, Janusz A, Kaczmarek L, Leigh MJ, Gabriel N, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. Am J Med Genet A 2013;161A:1897-1903.
89 Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet 2009;46:94-102.
90 Siller SS, Broadie K. Neural circuit architecture defects in a Drosophila model of Fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. Dis Model Mech 2011;4:673-685.
91 Leigh MJ, Nguyen DV, Mu Y, Winarni TI, Schneider A, Chechi T, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. J Dev Behav Pediatr 2013;34:147-155.
92 Pietrobono R, Pomponi MG, Tabolacci E, Oostra B, Chiurazzi P, Neri G. Quantitative analysis of DNA demethylation and transcriptional reactivation of the *FMR1* gene in fragile X cells treated with 5-azadeoxycytidine. Nucleic Acids Res 2002;30:3278-3285.
93 Torrioli MG, Vernacotola S, Peruzzi L, Tabolacci E, Mila M, Militerni R, et al. A double-blind, parallel, multicenter comparison of L-acetylcarnitine with placebo on the attention deficit hyperactivity disorder in fragile X syndrome boys. Am J Med Genet A 2008;146A:803-812.
94 Arnold LE, Amato A, Bozzolo H, Hollway J, Cook A, Ramadan Y, et al. Acetyl-L-carnitine (ALC) in attention-deficit/hyperactivity disorder: a multi-site, placebo-controlled pilot trial. J Child Adolesc Psychopharmacol 2007;17:791-802.

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