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Fragile X Syndrome and Targeted Treatment Trials

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Abstract

Work in recent years has revealed an abundance of possible new treatment targets for fragile X syndrome (FXS). The use of animal models, including the fragile X knockout mouse which manifests a phenotype very similar to FXS in humans, has resulted in great strides in this direction of research. The lack of Fragile X Mental Retardation Protein (FMRP) in FXS causes dysregulation and usually overexpression of a number of its target genes, which can cause imbalances of neurotransmission and deficits in synaptic plasticity. The use of metabotropic glutamate receptor (mGluR) blockers and gamma amino-butyric acid (GABA) agonists have been shown to be efficacious in reversing cellular and behavioral phenotypes, and restoring proper brain connectivity in the mouse and fly models. Proposed new pharmacological treatments and educational interventions are discussed in this chapter. In combination, these various targeted treatments show promising preliminary results in mitigating or even reversing the neurobiological abnormalities caused by loss of FMRP, with possible translational applications to other neurodevelopmental disorders including autism.

17.1 Introduction

We are in an age of targeted treatments for neurodevelopmental disorders that began with advances in neurobiology and the development of appropriate animal models for many neurodevelopmental disorders. Although the focus of this book and this chapter is on fragile X syndrome (FXS) and animal models for this disorder leading to targeted treatments, this is a phenomenon that has occurred for many other neurodevelopmental disorders including tuberous sclerosis (de Vries 2010), neurofibromatosis and other disorders of the RAS MEK pathways (Rauen et al. 2010), Down syndrome (Rueda et al. 2008), Rett syndrome

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(Maezawa and Jin 2010), and others with a known gene deletion or mutation. Targeted treatment strategies are only just beginning in autism, because it is a heterogeneous disorder with no single gene mutation causing the majority of cases (Bent and Hendren 2010). Most cases of autism involve abnormalities occurring in genes involved with synaptic plasticity, brain connectivity, and/or gamma amino butyric acid (GABA) and glutamate imbalances so that brain function is impaired (Belmonte and Bourgeron 2006; Kelleher and Bear 2008; Pinto et al. 2010). Thus, the autism field is benefitting from advances in targeted treatment for other disorders that are associated with autism, such as FXS, which is the most common single gene disorder associated with autism (Hagerman et al. 2010). One reason that FXS is a good model for autism is because FMRP is an RNA binding protein that transports, stabilizes, and regulates the translation of hundreds of mRNAs at the synapse (Darnell et al. 2005; Zalfa et al. 2007; Bassell and Warren 2008; Darnell et al. 2011; Luo et al. 2010). Not only does FMRP regulate many genes that when mutated lead to autism such as neuroligins, neurorexins, and SHANK proteins (Darnell et al. 2011; Hagerman et al. 2010), but the levels of FMRP in the brains of adult autistic patients have been documented to be low compared to controls even in individuals that do not have a fragile X mutation (Fatemi and Folsom 2010). Not only autism but other neuropsychiatric disorders have been reported to have low levels of FMRP in the CNS, including schizophrenia, severe depression, and bipolar disorder (Fatemi et al. 2010). Although FMRP controls the translation of many mRNAs it is likely there are many cellular mechanisms that control the levels of FMRP, particularly mechanisms associated with neuropsychiatric disorders. The commonalities across disorders is an exciting new finding among neurodevelopmental disorders because it means that therapies developed for one disorder are likely to be helpful for many other disorders (Wang et al. 2010b). Preliminary evidence described later suggests that the new targeted treatments for FXS will also be helpful for autism and perhaps other neuropsychiatric disorders.

In this chapter, we will review animal studies leading to targeted treatments and then review the studies in patients with FXS. Although a number of medications are currently available that are frequently utilized for treatment for FXS they are not considered targeted treatments for FXS because they do not reverse the neurobiological abnormalities of FXS but they are generally helpful for the common symptoms in a variety of neurodevelopmental disorders (for a more complete review, see Tranfaglia, Chap. 15). Stimulants are effective for treatment of ADHD in FXS (Hagerman et al. 1988; Berry-Kravis and Potanos 2004; Hagerman et al. 2009), selective serotonin reuptake inhibitors (SSRIs) are helpful for the pervasive anxiety in FXS (Hagerman et al. 1994; Berry-Kravis and Potanos 2004; Hagerman et al. 2009), and atypical antipsychotics are helpful for mood stabilization and treatment of aggression and irritability (Erickson et al. 2010b). These commonly used treatments have been reviewed elsewhere (Hagerman et al. 2009). The remaining sections of this chapter focus on targeted treatments for FXS; these are summarized in Table 17.1.

17.1.1 Developing Treatment Strategies for FXS Based on Fmr1-Knockout Mouse Studies

Mutations in the *FMR1* gene that lead to transcriptional silencing and loss of FMRP expression result in FXS. The Fmr1-Knockout (KO) mouse does not express FMRP, exhibits many of the phenotypic characteristics of the human FXS condition, and has been an extremely useful tool to investigate the nervous system abnormalities arising from the

loss of FMRP, as well as for the development of potential therapeutics for the treatment of this syndrome. Recent discoveries made from investigations using the Fmr1-KO have given rise to potential therapeutics for FXS, and in particular for the treatment of intellectual disability. As will be discussed, a number of approaches have found some success in the animal model with subsequent human trials. The animal models, to varying degrees, have led to positive outcomes for anatomical, electrophysiological, and behavioral measures across the different strategies.

17.1.1.1 Group I Metabotropic Glutamate Receptor Strategy—One of the first insights into the neurochemical underpinnings of FXS came from work by Bear, Huber, and colleagues on group I metabotropic glutamate receptors (mGluRs). In the hippocampal field CA1, activation of mGluR5 leads to long-term depression (LTD), which is seen as a reduction in synaptic responses. Importantly, LTD triggered by mGluR activation (mGluR-LTD) requires the rapid translation of preexisting mRNA in the postsynaptic dendrites (Huber et al. 2000). Huber et al. found in the Fmr1-KO that hippocampal LTD was more pronounced (greater depression) than in wild types (Huber et al. 2002). This work gave rise to the mGluR theory of Fragile X which argues that the psychiatric and neurological aspects of the syndrome are a consequence of an exaggerated response to group I mGluR1/5 activation (Bear et al. 2004); for more details as to role of FMRP in negatively regulating local protein synthesis, and how the lack of synthesis inhibition leads to exaggerated LTD, the reader is referred to several comprehensive reviews (Waung and Huber 2009; Berry-Kravis et al. 2011; Chap. 18). This work has compelled numerous investigations into the outcomes of blocking group I mGluRs, most particularly the mGluR5 subtype, in the Fmr1-KO on different aspects of the phenotype that align with the human condition.

Studies aimed at blocking mGluR5 have principally used the selective noncompetitive antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) (Gasparini et al. 1999). Reducing mGluR5 function either with MPEP treatment or by lowering mGluR5 levels (~50%) in the Fmr1-KO has been shown to ameliorate a number of abnormal features in the mouse model that, to a great degree, reflect the human FXS phenotype. These features in the mouse mutant and the effects of mGluR5 antagonism/reduction on them are described later.

Dendritic Spine Morphology: Dendritic spine abnormalities have been described in both FXS (Rudelli et al. 1985; Hinton et al. 1991; Wisniewski et al. 1991; Irwin et al. 2001) and the Fmr1-KO (Comery et al. 1997; Irwin et al. 2002; Grossman et al. 2006). By and large, the fragile X mutation results in greater spine density on adult cortical neurons and greater numbers of spines that have an “immature” profile: There is a lower density of mushroom shaped spines with large heads, a greater number of longer spines, and excessive filopodia. In both developing and adult hippocampal neurons, spine abnormalities are present (Braun and Segal 2000; Antar et al. 2006; Grossman et al. 2006; de Vrij et al. 2008; Bilousova et al. 2009). Correcting these defects in spine morphology and numbers has become a standard “litmus test” in the field for evaluating the efficacy of drug treatments. Thus far, spine abnormalities seen in hippocampal neurons in vitro have been rescued with two independent mGluR5 antagonists, MPEP and fenobam (de Vrij et al. 2008). In addition, reducing mGluR5 expression in brain normalized spine density on visual cortical neurons in the adult

animal (Dölen et al.2007). While more work is needed to examine effects on spine morphology in the adult brain, these data suggest that mGluR5 antagonism in FXS could be very beneficial.

Protein Synthesis: FMRP binds to mRNAs (including its own) and regulates their translation within dendrites and spines in response to neural activation (Weiler et al. 1997, 2004) and, in particular, occurs in response to activation by either group I mGluR, (Huber et al. 2002; Antar et al. 2004; Aschrafi et al. 2005), muscarinic (M1) acetylcholine receptors (Volk et al. 2007), and possibly other synaptic Gq-linked receptors including dopamine D1 receptors (Wang et al. 2010a). In the Fmr1-KO, levels of synaptic proteins for a number of FMRP target mRNAs are elevated including MAP1B, PSD95, CaMKII, APP, Arc, and PP2A, amongst others [reviewed in (Berry-Kravis et al. (2011)]. In vivo treatment of Fmr1-KOs with MPEP has been shown to increase levels of mRNA granules (levels are reduced in the mutant as a consequence of heightened translation) indicating that mGluR5 antagonism can normalize protein synthesis in the KO (Aschrafi et al. 2005). Overall protein synthesis in Fmr1-KO brain also is reduced by lowering mGluR5 levels by half (Dölen et al. 2007), further supporting the idea that blockade of this receptor subtype will normalize protein content in FXS.

Long-term Depression: As described earlier, hippocampal mGluR-dependent LTD is more pronounced in the Fmr1-KO as compared to wild-type mice (Huber et al. 2002; Hou et al. 2006; Nosyreva and Huber 2006; Sharma et al. 2010); as induction of this type of synaptic plasticity is dependent on group I mGluR activation, tests of antagonism of this receptor group have not been conducted. Interestingly though, a recent study by Choi et al. (2010) showed that chronic treatment (8 weeks) with the group II mGluR antagonist LY341495 in Fmr1-KOs reduced their level of hippocampal mGluR-LTD to near wild-type levels suggesting that targeting other mGluRs may also be beneficial. Finally, enhanced LTD has been reported in Fmr1-KO cerebellum as well (Koekkoek et al. 2005), although group I mGluR antagonists have not been tested in this system.

Long-term Potentiation: Long-term potentiation (LTP) reflects greater synaptic strength, and is considered the main cellular substrate thought to underlie learning and memory. In the Fmr1-KO, deficits in LTP have been reported for a number of brain regions including the neocortex (Li et al. 2002; Meredith et al. 2007; Wilson and Cox 2007), the piriform cortex (Larson et al. 2005), the hippocampus (Lauterborn et al. 2007; Shang et al. 2009; Chen et al. 2010), and the amygdala (Zhao et al. 2005; Suvrathan et al. 2010). In the few brain areas surveyed thus far mGluR5 antagonism by MPEP does not correct this aspect of the phenotype (Wilson and Cox 2007; Suvrathan et al. 2010), although this drug has been reported to rescue spontaneous excitatory postsynaptic currents in the Fmr1-KO (Suvrathan et al. 2010; Meredith et al. 2011). These data suggest that some, but not all, synaptic defects may be amenable to group I mGluR-targeted intervention.

Prepulse Inhibition: One of the most common clinical features of FXS is heightened sensitivity to sensory stimulation (Cohen 1995; Miller et al. 1999; Frankland et al. 2004). Prepulse inhibition (PPI) of an acoustic startle response, a widely used model to study basic

sensorimotor processing, has been shown to be related to mGluR signaling (Grauer and Marquis 1999). While PPI is reportedly reduced in humans with the fragile X full mutation (Hessl et al. 2008), studies in the Fmr1-KO are mixed with one group reporting enhanced (Frankland et al. 2004) and another group reporting reduced (de Vrij et al. 2008) PPI, albeit with PPI measurement conducted differently in these two studies. Interestingly, the defect in the Fmr1-KO's PPI response reported by de Vrij et al. (2008) was measured via a similar protocol to that used in humans with FXS and was rescued by MPEP treatment (de Vrij et al. 2008).

Seizures: A substantial number (~15%) of patients with FXS suffer from epilepsy during development (Musumeci et al. 1999; Sabaratnam et al. 2001; Berry-Kravis et al. 2010). While the factors responsible for this hyperexcitability in FXS are poorly understood, enhanced Gp1 mGluR activation has been shown to induce epilepti-form activity (Ure et al. 2006; Karr et al. 2010). As in the human condition, loss of FMRP in the mouse model results in a greater tendency towards seizures and, in particular, Fmr1-KOs have a more excitable audiogenic seizure pathway (Chen and Toth 2001; Yan et al. 2005; Musumeci et al. 2007), and more protracted hippocampal seizures following kindling (Qiu et al. 2009), than do wild types. Treatment with MPEP has been shown to suppress both audiogenic and limbic seizures in the KO (Yan et al. 2005; Qiu et al. 2009), and reducing mGluR5 levels by 50% also significantly attenuated audiogenic seizures (Dölen et al. 2007). Similarly, studies of the hippocampus have shown that endogenous glutamatergic transmission induces prolonged synchronized discharges in KOs but not in wild types, suggesting a greater degree of excitability in the mutant (Chuang et al. 2005). This effect in Fmr1-KOs was mediated by Group I mGluRs as it was blocked by both mGluR5 (MPEP) and mGluR1 (LY367385) antagonists. As to the downstream mechanism involved in the induction of prolonged synchronized discharges, previous work has implicated the extracellular signal-regulated kinase 1/2 (ERK1/2; a.k.a. MAPK) (Zhao et al. 2004). Inhibition of ERK signaling in the Fmr1-KO hippocampus with a mitogen-activated protein kinase kinase (MEK) inhibitor also blocked the prolonged synchronized discharges (Chuang et al. 2005).

Learning: While learning deficits have been difficult to reliably assess in the Fmr1-KO perhaps due to strain differences, a number of studies have described learning problems for the mutant in different tasks including Morris water maze (Bakker and Consortium 1994; D'Hooge et al. 1997; Dobkin et al. 2000), radial maze (Mineur et al. 2002), fear conditioning (Paradee et al. 1999; Qin et al. 2005), object discrimination (Ventura et al. 2004), odor discrimination (Larson et al. 2008), and eye blink conditioning (Koekkoek et al. 2005). Surprisingly, little work has been done to test the effect of group I mGluR antagonism on learning in the Fmr1-KO. To date, Dölen et al. (2007) have shown that genetic reduction of brain mGluR5 levels rescues inhibitory avoidance (extinction) learning in the Fmr1-KO. While further studies are needed to assess mGluR5 antagonists on this aspect of the phenotype in mammals, studies in the *Drosophila* (*dfmr*) model of FXS have shown positive effects of MPEP treatment on learning (McBride et al. 2005; Bolduc et al. 2008).

Motor Behavior: Fmr1-KOs have been reported to have abnormal motor behavior including displaying hyperactivity (Bakker and Consortium 1994; Mineur et al. 2002; Qin et al. 2005; Restivo et al. 2005), increased exploratory behavior (Bakker and Consortium 1994), and spending more time in the center of an open field (Yan et al. 2004; Qin et al. 2005). Treatment with MPEP has been shown to reduce center field behavior in the KO to one indistinguishable from wild type (Yan et al. 2005), but effects of mGluR5 antagonism on other motor behaviors have not been assessed.

Macroorchidism: As in the human FXS condition, postadolescent male Fmr1-KOs have enlarged testes (macroorchidism) (Bakker and Consortium 1994; Kooy et al. 1996; Yan et al. 2004). Neither partial reduction nor full loss of mGluR5 expression in the Fmr1-KO rescues this aspect of the phenotype (Dölen et al. 2007).

Autism-Like Behaviors: The above studies indicate that Group I mGluR antagonism in patients with FXS could have substantial effects across a wide range of clinical features in this syndrome. Importantly though, one aspect of the syndrome that has yet to be addressed in the mouse model, or with mGluR5 antagonism in particular, is autism. About 30% of individuals with FXS are diagnosed with autism, a disorder characterized by abnormal reciprocal social interactions, communications deficits, and repetitive behaviors. While autistic-like behaviors have yet to be fully investigated in the Fmr1-KO they do display some social behavioral abnormalities (Mineur et al. 2006; McNaughton et al. 2008). By comparison, the inbred mouse strain BTBR T + tf/J (BTBR) has been investigated to a greater extent and reported to have a number of features associated with autism (McFarlane et al. 2008). Treatment of BTBR mice with MPEP improves some aspects of their behavior such as reducing repetitive grooming, but does not improve their sociability (Silverman et al. 2010). Further work is needed in both mouse models and human testing to evaluate whether mGluR5 antagonism is effective in the treatment of autism-related behaviors.

17.1.2 mGluR5 Antagonists in Human Trials

The first trial of an mGluR5 antagonist in patients with FXS, sponsored by Neuropharm LTD, involved the use of fenobam in 12 adults with FXS given a single dose (Berry-Kravis et al. 2009). Although the purpose of this single-dose trial was to assess pharmacokinetics and side effects, there was a positive behavioral response with improved communication and eye contact in addition to improvement in the PPI deficit which has been documented in patients with FXS (Hessl et al. 2008). Although this first trial of fenobam was very promising, further development of fenobam was not pursued by Neuropharm due to financial challenges.

The next study of an mGluR5 antagonist in adults with FXS was a European trial of AFQ056 that took place at three centers (Jacquemont et al. 2011). This study was double blind and included 30 patients with FXS, ages 18–35, who were randomized to AFQ056 or placebo, underwent dose up titration, 14 days of full dose treatment, and then down titration (total treatment period 28 days) and then crossed over after a 1-week washout period between treatment sessions. Although the overall patient cohort did not demonstrate efficacy of AFQ056 compared to placebo in the primary measures, there was a positive response to

AFQ056 on the Repetitive Behaviors Scale (RBS-R). In an exploratory analysis it was found that those patients who were fully methylated (n = 7) demonstrated a significant response to AFQ056 in the primary outcome measures, the Aberrant Behavior Checklist (ABC), and the Clinical Global Impression Scale (CGI) in addition to most of the secondary outcome measures compared to placebo (Jacquemont et al. 2011). This demonstrates a methylation biomarker for drug response, most likely reflective of clinical response after short-term treatment in those that are more affected by FXS.

Other mGluR5 antagonists are being assessed currently in multicenter clinical trials including R04917523 (Roche Pharmaceuticals) and also STX107 (Seaside Therapeutics). Further study will be needed to know if these agents are efficacious in FXS compared to placebo.

17.1.2.1 Targeting GABA Receptors—Work on both the mouse and fly models of FXS demonstrate that they have lower levels of GABA receptors, with the Fmr1-KO exhibiting clear reductions in the GABA-A subtype in brain (El Idrissi et al. 2005; D'Hulst et al. 2006; Gantois et al. 2006). In addition, the Fmr1-KO exhibits reduced inhibitory postsynaptic currents in the amygdala (Olmos-Serrano et al. 2010) and abnormal GABA-A currents in subicular neurons (Curia et al. 2008), but levels of glutamic acid decarboxylase, the rate limiting enzyme for GABA synthesis, in brain are mixed (El Idrissi et al. 2009; Olmos-Serrano et al. 2010). As GABA is the principal inhibitory neurotransmitter of the CNS, the collective findings indicate that the balance between neuronal inhibition and excitation in FXS would favor more overall excitation; this conclusion is consistent with the observation that seizures are more prevalent in FXS than in the general population. Two approaches for restoring appropriate levels of GABA-mediated inhibition entail use of agonists to either the GABA-A or GABA-B receptor subtypes. GABA-A agonists act to directly compensate for the GABA-A subunit deficiencies, whereas GABA-B agonists act presynaptically to block glutamate release thus decreasing glutamatergic drive in general, but also would be expected to reduce group I mGluR activation and downstream signaling events. In the Fmr1-KO, the GABA-A agonists ganaxolone and taurine have been reported to reduce audiogenic seizures (Kooy et al. 2010) and improve learning in a passive avoidance test (El Idrissi et al. 2009), respectively. The GABA-B agonist R-baclofen (Arbaclofen; the right-sided enantiomer of baclofen) also rescues the audiogenic phenotype in the mouse model (Pacey et al. 2009), and normalizes several behaviors including marble burying and open field locomotor activity (Paylor 2008). Similarly, studies in the *dfmr* mutant fly show that a variety of GABA agonists ameloriate the lethality phenotype from glutamate-containing food, neuropathology, excessive protein translation, and abnormal courtship behavior (Chang et al. 2008).

17.1.2.2 Arbaclofen Trials in Individuals with FXS—In addition to animal data discussed earlier, anecdotal clinical experience suggesting behavioral benefits from racemic baclofen administered to patients with autism and fragile X in a clinical setting, and data from TMS studies demonstrating enhancement of cortical inhibition by racemic baclofen (McDonnell et al. 2007), supported the concept of baclofen as a possible treatment for humans with FXS. Arbaclofen (R-baclofen) has more potent GABA-B agonist activity,

leading to development of this molecule for the treatment of FXS. An initial pilot double-blind placebo-controlled crossover trial of arbaclofen for children and adults with FXS, age 6–40 years, conducted by Seaside Therapeutics, involved 4-week periods of placebo and active drug treatment for each subject, with drug washout in between treatment periods. This trial showed benefit for arbaclofen over a placebo in global preference for the treatment period and clinician global impression, and was particularly evident in the subgroups of FXS patients with autism, more severe irritable behavior, or more severe social deficits. In the group with more impairment in social behaviors (ABC Social Withdrawal Score >8), significant improvement on the ABC Social Withdrawal scale, Vineland Play and Leisure Scale, and Visual Analog Scale rating for behavior were also seen (Wang et al. 2011), as well as a significantly increased number of responders ("much" or "very much" improved on the CGI and a > 25% improvement on the ABC Social Withdrawal subscale) during arbaclofen as opposed to placebo treatment. There were no significant safety issues and a very mild side effect profile. Many subjects are continuing treatment though an extension study, to evaluate the long-term benefits and the toxicity. Anecdotally, many of these subjects continue to show benefits of treatment and further development of arbaclofen is in progress with additional clinical trials pending.

17.1.2.3 Ampakines and Targeting Brain-Derived Neurotrophic Factor—The neurotrophin brain derived neurotrophic factor (BDNF) has been shown in numerous studies to be a positive modulator of synaptic plasticity. In particular, application of BDNF or increasing endogenous levels of BDNF production facilitates hippocampal LTP, as well as memory (Kramár et al. 2004; Minichiello 2009). Recent work has shown that BDNF corrects hippocampal LTP deficits in several rodent models of diseases or conditions that are characterized by memory impairment, including those for Huntington's disease (Lynch et al. 2007; Simmons et al. 2009), middle aging (Rex et al. 2006), and menopause (Kramar et al. 2010). Similarly, BDNF was also tested in the Fmr1-KO to determine if the neurotrophin could restore hippocampal LTP in the mutant: Using theta burst stimulation (TBS) to elicit LTP in the hippocampal CA1 region, the Fmr1-KOs were found to have a higher threshold of induction such that five theta bursts only induced LTP in WT hippocampal slices and not in Fmr1-KOs. However, in the presence of BDNF (nM) five theta bursts elicited LTP in Fmr1-KO slices to the same degree as seen in WT slices (Lauterborn et al. 2007). The fact that BDNF corrected the deficit does not, in and of itself, indicate that BDNF levels are perturbed in the Fmr1-KO. In fact, protein measures for both BDNF and its high affinity receptor TrkB in hippocampus were comparable between KOs and wildtypes. However, recent work by Louhivuori et al. (2011) in the Fmr1-KO has shown that BDNF mRNA is mis-localized in neocortical and hippocampal neurons suggesting that the site(s) of neurotrophin release and signaling may be abnormal. Furthermore, Selby et al. (Selby et al. 2007) have reported that TrkB levels are higher in a subgroup of neocortical GABAergic interneurons suggesting that cell-type specific alterations in the receptor may be present in FXS. Further work is needed to determine if disturbances in BDNF release, and thus availability at the synapse, are present in the Fmr1-KO and if the responsivity of the TrkB receptor is abnormal.

One would predict that drugs that augment BDNF content in brain likely facilitate learning and memory. A class of drugs that does both (increases BDNF expression and enhances learning) is the positive AMPA receptor modulators, also known as “ampakines.” Ampakines enhance fast, excitatory transmission at central synapses (Staubli et al. 1994a, b), and produce a variety of acute effects including lowered thresholds for LTP and accelerated learning in animals ((Lynch and Gall 2006) for review); effects on memory encoding in humans also have been reported (Ingvar et al. 1997). Ampakines also increase the expression of BDNF in hippocampal and neocortical neurons, both in vitro and in vivo, with elevated levels of BDNF lasting for days following a single injection/treatment (Lauterborn et al. 2000; Legutko et al. 2001; Lauterborn et al. 2003, 2009). Importantly, ampakine-induced increases in BDNF are neuroprotective in models of insult (Destot-Wong et al. 2009; Jourdi et al. 2009a), and can facilitate both LTP (Rex et al. 2006; Simmons et al. 2009; Kramar et al. 2010) and behavior (Simmons et al. 2009) in different animal models of cognitive impairment. Thus, the overall ampakine strategy for the treatment of cognitive impairment in FXS should be viewed as having two facets: an immediate effect of the ampakine on AMPAR function and a more protracted effect on synaptic plasticity through longer term effects on BDNF content. As expression of AMPA receptors is reduced in many brain regions of the Fmr1-KO (Li et al. 2002; Muddashetty et al. 2007; Suvrathan et al. 2010), direct positive modulation of residual receptors could be very beneficial for enhancing glutamatergic-mediated synaptic plasticity. While studies are ongoing to assess the ampakines for effects on LTP, spine morphology, and cognitive behavior in the Fmr1-KO, it is important to note that the ampakines effectively increase BDNF expression in this animal model (Lauterborn and Gall 2004) making it possible to test the long-term effects of enhanced neurotrophism on its phenotype.

Finally, a significant finding in the Fmr1-KO is the enhanced internalization of AMPA receptors in this mutant by mechanisms engaged by at least two different receptors, mGluR5 and dopamine D1 (Nakamoto et al. 2007; Wang et al. 2010a). Importantly, mGluR5 antagonism has been shown to block the internalization of the AMPARs (Nakamoto et al. 2007). Thus, it seems reasonable to conclude that combinational therapy with both an mGluR5 antagonist, which increases AMPAR levels at the synapse and reduces exaggerated protein synthesis, and an ampakine, which facilitates synaptic plasticity and enhances neurotrophism, could be particularly efficacious as a treatment strategy for the cognitive and behavioral problems in FXS.

17.1.2.4 Use of Ampakines in FXS—A single human trial has been completed with CX516 (Cortex Phamaceuticals), a direct AMPA receptor positive modulator known to increase LTP and raise BDNF levels (Jourdi et al. 2009b). This was a double-blind placebo-controlled trial of effects of CX516 on the safety and the cognitive and behavioral efficacy measures carried out in a cohort of 49 individuals with FXS (Berry-Kravis et al. 2006). The primary outcome measure was a *z*-score for memory across several verbal and nonverbal memory tasks. Conceptually, it was thought the CX516 would help compensate or correct the AMPA receptor deficit resulting from mGluR pathway overactivity. Realistically, CX516 is a very weak ampakine and provides only weak BDNF induction, and thus no improvement was seen in the primary outcome measure of memory, nor were any other

behavioral or cognitive improvements observed across the full subject group. Improvement in global functioning was seen in the subgroup of five patients co-treated with an antipsychotic (known to potentiate ampakine activity), relative to the four patients on placebo and an antipsychotic. This suggests that a more potent ampakine molecule might be successful in treating FXS; however, such molecules have not yet come to clinical trials.

Although this trial did not produce the desired improvement in functioning, there were no major safety issues, providing encouragement for future use of more potent AMPA activators in the FXS population. Further, this trial was the first to demonstrate that large fairly intensive phase II clinical trial could be successfully performed in groups of subjects with FXS, with high completion rates for study procedures.

17.1.2.5 The Dopaminergic System and Stimulants—Individuals with FXS often display hyperactivity, attention deficit, and lack of impulse control (Hagerman and Silverman 1991). Dysfunction in frontal-subcortical circuits (i.e., reduced dopaminergic drive) is thought to give rise to these types of behavior (Hjalgrim et al. 1999), and stimulants that modulate forebrain dopaminergic tone correct them (Solanto 2002). Consistent with this, recent work by Fulks et al. (2010) demonstrated that the Fmr1-KOs have reduced extracellular dopamine levels in striatum. Increased dopamine turnover in the cortical regions, the striatum, and the hippocampus also has been reported for the KO (Gruss and Braun 2004). In addition, dopamine receptor 1 (D1) signaling is impaired in both the striatum and the prefrontal cortex of the mutant, and treatment of Fmr1-KO mice with the D1 receptor agonist SKF81297 partially reversed their hyperactive locomotor activity and enhanced their motor function on the rotarod apparatus (Wang et al. 2008a). The psychostimulant amphetamine has also been shown to elicit a greater increase in dopamine release in the prefrontal cortex of Fmr1-KOs as compared to wild-type mice, and improved their ability to discriminate objects (Ventura et al. 2004), suggesting that stimulants may be useful for restoring some balance in dopaminergic tone in forebrain and improving behavior in FXS.

17.1.2.6 Human Studies of Stimulants and Aripiprazole in FXS—There has only been one controlled trial of stimulants in children with FXS and it demonstrated that two-thirds of the patients responded well to the stimulant compared to the placebo (Hagerman et al. 1988). Stimulants are widely used now in children with FXS who are 5 years or older and the effect is generally positive with improvement in hyperactivity and attention (Amaria et al. 2001; Berry-Kravis and Potanos 2004; Hagerman et al. 2009). Occasionally on a higher dose greater activation or a lower number of verbalizations can be problematic but stimulants are usually well tolerated. A negative response to stimulants in a patient under 5 years of age should not deter a trial after 5 years since this drug class is more likely to be tolerated and effective after age five.

Although aripiprazole is a treatment directed primarily at behavior rather than specific molecular mechanisms, it could be theoretically targeted to dopamine deficits described in the *fmr1* knockout mouse (Wang et al. 2008a, b), given its dopamine agonist activity at lower doses. Apipirazole has shown good success when used empirically in FXS clinic populations (Berry-Kravis and Potanos 2004; Hagerman et al. 2009) and resulted in

improvement in the ABC Irritability score, other ABC subscores, and additional behavioral rating scales in 15 participants with FXS treated in a very recently completed open-label trial (Erickson et al. 2010b). Initiation of a double-blind placebo-controlled trial of aripiprazole is planned.

17.1.2.7 Targeting Proteins that Regulate the Spine Actin Cytoskeleton—The spine actin cytoskeleton is a dynamic network that supports the shape, and ultimately the function, of the postsynaptic structure. There are numerous proteins and signaling pathways that act to regulate the actin cytoskeleton including the Rho GTPases, and mutations in a number of these proteins have been associated with different forms of mental retardation [reviewed in (van Galen and Ramakers 2005)]. Recent work in *Drosophila* has shown that dfmr (the fly homologue of FMRP) binds to the mRNA encoding the small Rho GTPase dRac (Lee et al. 2003), suggesting that FMRP regulates proteins critical to actin remodeling. Rac signals through its downstream effector p21-activated kinase (PAK), a family of serine–threonine kinases comprised of at least three members, PAK1, PAK2, and PAK3. The Rac–PAK pathway recently has been shown to be important for the stabilization of newly formed actin filaments that occur following TBS (Rex et al. 2009). Although loss-of-function mutations in the *PAK3* gene are associated with non-syndromic X-linked mental retardation (Allen et al. 1998; Bienvenu et al. 2000), recent work from Hayashi and colleagues (2007) has suggested that excessive PAK activity in Fmr1-KOs may be an underlying cause of the dendritic spine abnormalities. In particular, these authors demonstrated that spine abnormalities in neocortex were partially ameliorated in Fmr1-KOs that expressed a dominant negative *PAK* transgene in the forebrain (Hayashi et al. 2007). Likewise, cortical LTP was fully restored in the Fmr1-KO by reduced PAK expression. Finally, several behavioral abnormalities, including locomotor activity, stereotypy, anxiety, and trace fear conditioning, in the KOs also were ameliorated to some degree by the dominant negative *PAK* transgene. These data suggest that inhibition of PAK activity could be a potentially interesting therapeutic target for aspects of the FXS phenotype. To this end, PAK inhibitors are being developed and in initial testing have shown that they correct spine defects and restore LTP in the neocortex (Vollrath et al. 2010).

With regard to the PAK inhibitors, it is important to note that systemic use of these compounds may still only result in regionally selective effects in brain: Although Hayashi et al. were able to attain reduced PAK levels in both the neocortex and the hippocampus the effect on dendritic spine features was only observed in neocortex (Hayashi et al. 2004). Moreover, recent work by Chen and colleagues (2010) in the Fmr1-KO hippocampus demonstrated that the physiological activation of both Rac and PAK in spines is deficient and, consistent with this, the newly polymerized spine actin that occurs following LTP-producing stimulation fails to properly stabilize. These data suggest that the consequence of FMRP loss on RAC–PAK pathway signaling may be different between the cortex and the hippocampus, and that the use of PAK inhibitors may be regionally effective for certain aspects of the FXS phenotype. Evaluation of the impact of these compounds on the different forms of memory (i.e., those ascribed to hippocampus versus other structures) will be particularly interesting.

17.1.2.8 Targeting Other Intracellular Signaling Pathways: Phosphatase and Kinase Inhibitors—Several signaling pathways that regulate protein translation are perturbed in the Fmr1-KO. In particular, mGluR-dependent translation occurs through two major signaling pathways, the ERK–MAPK and PI3 Kinase-mTOR pathways, with convergence on the translation initiation (eIF4F) complex [reviewed in(Waung and Huber 2009)]; inhibition of either PI3 kinase, mTOR, ERK, or translation initiation itself prevents mGluR-LTD (Huber et al. 2000; Gallagher et al. 2004; Hou and Klann 2004). Studies in the Fmr1-KO have shown that the activation of both ERK and mTOR is misregulated (Kim et al. 2008; Weng et al. 2008; Sharma et al. 2010), consistent with the observation that protein synthesis in the mutant is aberrant. Moreover, other proteins that control gene expression and other cellular processes are also misregulated in the KO including glycogen synthase kinase-3β (GSK3β) (Min et al. 2009; Yuskaitis et al. 2010a). As such, a number of studies have targeted these systems (amongst others) and the results for specific drugs and/or targets are encouraging.

Phosphatase Inhibitors: Weng et al. showed that the phosphorylation of ERK in both neurons and thymocytes of Fmr-1 KOs, and in lymphocytes from peripheral blood of individuals with FXS, is delayed (Weng et al. 2008). Kim et al. (2008) also demonstrated that Group I mGluR-dependent activation of the ERK pathway in the Fmr1-KO is abnormal. Specifically, following mGluR1/5 stimulation ERK is phosphorylated in wild-type cortical synaptoneurosomes but *dephosphorylated* in KO cortical synaptoneurosomes (Kim et al. 2008). These results suggest that in response to synaptic stimulation there is aberrant activation of phosphatases in Fmr1-KO synapses. In agreement with this, both protein phosphatase 2A (PP2A) and tyrosine phosphatase were found to be overactivated after mGluR1 and mGluR5 stimulation, respectively, resulting in the rapid deactivation of ERK in Fmr1-KO samples. Pretreatment with a PP2A blocker, however, fully restored ERK activation in Fmr1-KO synaptoneurosomes. The consequence of overactive phosphatases and a misregulated ERK pathway in FXS could be multifold as the MAPK/ERK pathway is involved in many cellular processes. However, it is important to note that not all aspects of the FXS phenotype may be ameliorated by facilitating ERK activation as Chuang et al. (2005) showed that *inhibition* of ERK signaling in the Fmr1-KO was beneficial for controlling seizure-like activity.

PI3K Inhibitors: Recent work by Gross et al. (2010) has shown that PI3K activity, and downstream signaling to Akt, is markedly increased in Fmr1-KO synapses (Gross et al. 2010). Interestingly, this elevation in PI3K activity is dependent upon the absence of FMRP but not on the presence of group I mGluRs, although mGluR5 antagonism corrected it (Gross et al. 2010). Antagonism of PI3K signaling with two different drugs, LY294002 and wortmannin, rescued excessive synaptic translation in the KO (Gross et al. 2010); treatment with rapamycin, which inhibits the PI3K downstream signaling molecule mTOR, also reduced translation in the KO and is in line with the observation of increased phosphorylation and activity of mTOR in the absence of FMRP (Sharma et al. 2010). Finally, the same group showed that treatment of *Fmr1* knockout neurons in culture with the PI3K inhibitor LY294002 in vitro reduced AMPAR endocytosis and normalized protrusion (including spines and filopodia) density in Fmr1-KO neurons to WT levels (Gross et al.

2010). These data are intriguing in that they further support the idea of selectively targeting the PI3K-mTOR pathway for the treatment of FXS. Currently, inhibitors of this pathway are being investigated in preclinical models of cancer with some success (McMillin et al. 2009), suggesting the possibility that selective compounds could be available for testing in other disorders such as FXS in the future.

GSKβ Inhibition and Lithium: Lithium is principally used to treat mood disorders and, although the exact mechanism is not understood, likely improves behavior through modulatory effects on various brain chemical systems including serotonin, dopamine, and the neurotrophin BDNF [reviewed in (Bschor et al. 2003; Beaulieu and Caron 2008; Gold et al. 2010)]. In recent years, work has more directly linked the effect of lithium to inhibition of GSK3β, a serine/threonine protein kinase, which in turn promotes β-catenin-dependent gene expression (Wada 2009). Work by Jope and colleagues has shown that the inhibitory form of GSK3β is reduced in the Fmr1-KO brain, liver, and testes, suggesting that this kinase is constitutively overactive in the mutant, and that lithium treatment normalizes these measures (Min et al. 2009; Yuskaitis et al. 2010a, b). Furthermore, lithium treatment recently has been shown to normalize levels of activated ERK (Venkitaramani et al. 2010), indicating that this drug is having effects across several protein synthesis-dependent pathways. In addition, recent work by Choi and colleagues demonstrate that lithium can restore normal mGluR-dependent LTD (Choi et al. 2010). Finally, lithium has been shown to reverse a number of behavioral abnormalities in the Fmr1-KO including open field hyperactivity (Min et al. 2009; Liu et al. 2010; Yuskaitis et al. 2010b), deficits on a social interaction task [(Liu et al. 2010; Mines et al. 2010), learning deficits (Liu et al. 2010; Yuskaitis et al. 2010b), anxiety (Liu et al. 2010; Yuskaitis et al. 2010b), novel object recognition (Venkitaramani et al. 2010), audiogenic seizures (Min et al. 2009), as well as dendritic spine shape (Liu et al. 2010), and macroorchidism (Yuskaitis et al. 2010a). Other GSK3 β inhibitors such as SB-216763 have been shown to reverse a number of these phenotypes as well (Min et al. 2009). Importantly though, the effects of GSK3β inhibitors and mGluR5 blockers are not additive, providing strong evidence that excess GSK3β activity is a direct consequence of excessive mGluR activity (Min et al. 2009).

17.1.2.9 Human Trials of Lithium in FXS—Although a number of intracellular treatment targets have been proposed, including lithium, PI3K inhibitors, GSK3β inhibitors, and PAK inhibitors, in most cases safe and available agents acting on these targets are not yet developed for use in humans. One exception is lithium, for which the preclinical findings in the *dfmr* mutant fly and *fmr1* knockout mouse, as described earlier, suggested promise of therapeutic benefit. Lithium may attenuate activation of the phospholipase C (PL-C) signaling pathway by inhibiting phosphatidyl inositol (PI) turnover (Berridge 1993), and clearly inhibits GSK3β activity (Min et al. 2009; Yuskaitis et al. 2010b) which would decrease phosphorylation of ERK and multiple signaling molecules that regulate translation; all of these effects would theoretically lead to reduction of translational activation. Given that lithium treatment does in fact normalize levels of activated ERK and GSK3β in the *fmr1* knockout (Venkitaramani et al. 2010), it appears that the main effect of lithium in the *fmr1* knockout is to reduce excessive GSK3β activity with resultant reduction in excessive ERK-mediated translation; however, lithium may also directly increase surface expression of

AMPA receptors (Du et al. 2010) and reduce excess MAP1B activity (Owen and Gordon-Weeks 2003).

Although lithium has been used for some time to treat mood instability and aggression in FXS (Berry-Kravis and Potanos 2004; Hagerman et al. 2009; Wang et al. 2010b), only anecdotal information on effectiveness existed, prior to a pilot proof-of-concept study initiated by Berry-Kravis et al. (2008a, b) to evaluate the strategy of inhibition of mGluR-activated translational signaling pathways as a treatment for FXS, by systematically exploring the effects of short-term (2 month) treatment with lithium on a broad range of phenotypes including behavior, cognition, and biophysical measures in a small cohort of subjects with FXS. In addition, since ERK (extracellular-signal regulated kinase) was shown to have a reduced rate of activation in the *fmr1* knockout and in lymphocytes from humans with FXS (Weng et al. 2008), ERK activation was explored as a potential biomarker for effects of lithium on cellular signaling and more generally as a model for measuring changes in signaling during treatment with agents that may impact receptor-activated translational regulatory pathways. In this pilot open-label trial in 15 patients with FXS (Berry-Kravis et al. 2008a), lithium treatment resulted in a significant improvement in behavior as was seen in on the Total Aberrant Behavior Checklist-Community Edition (ABC-C) Score, and the Hyperactivity, Inappropriate Speech, and Lethargy (Social Withdrawal) subscales of the ABC, the Maladaptive Behavior subscore from the Vineland Adaptive Behavior Scale (VABS), a parent visual analog scale for target behaviors, and the Clinical Global Impression (CGI) Scale. Improvement in verbal memory on the RBANS List Learning task was also demonstrated in addition to normalization of abnormal ERK phosphorylation rates in lymphocytes (Berry-Kravis et al. 2008a, b). There were no major side effects but polydipsia and polyuria were seen relatively frequently as expected, and there were a few subjects with abnormal thyroid measurements on lithium. A subgroup of 11 subjects continued on lithium for a year with persistent improvements in behavior on the ABC-C and VABS, and ongoing normalization of the ERK activation biomarker (Berry-Kravis, unpublished data), suggesting the behavioral improvements were less likely to be placebo effects. These data indicated that further studies with a placebo-controlled trial would be indicated, however such studies have not yet been carried out, partly due to concerns about the chronic toxicity of lithium, but also related to hope that less toxic mechanism-based treatments will be available soon.

17.1.2.10 Minocycline and Metalloproteinases—Minocycline is a broad-spectrum tetracycline analogue commonly used to treat acne and other skin diseases. Interest in this drug as a potential therapeutic for CNS disorders began with select studies showing that minocycline was neuroprotective in several mouse models of neurodegenerative disorders including Huntington's disease (Chen et al. 2000) and Alzheimer's disease (Choi et al. 2007). Recently, minocycline was tested in the Fmr1-KO for effects on hippocampal dendritic spine development and behavior (Bilousova et al. 2009). Bilousova et al. (2009) found that minocycline promotes the maturation of hippocampal dendritic spines in young neurons. Specifically, minocycline treatment of cultured Fmr1-KO hippocampal cells resulted in a greater proportion of mushroom shaped spines, thought to reflect more mature spines. While there was no effect of treatment on spine length or density, minocycline did

reduce the number of filopodia-like protrusions. Further, minocycline treatment of nursing dams beginning at time of birth for 1 week increased the proportion of hippocampal spines with larger heads and reduced the number of filopdia in the Fmr1-KO pups. While these findings are very encouraging and indicate that treatments with this drug could begin very early in development, tests of minocycline on other aspects of the phenotype including examination of the neocortex where the spine abnormalities are greater and persist through adulthood will be very important.

The mechanism(s) by which minocycline "normalizes" the maturation of hippocampal spines is not known but evidence suggests that it could be through regulation of matrix metalloproteinases (MMPs), which are zinc-dependent endopeptidases that degrade extracellular matrix proteins. Treatment of wild-type hippocampal neurons with either MMP-7 or MMP-9 results in a more immature dendritic spine phenotype (more filopodia and fewer mushroom-like spines) (Bilousova et al. 2006, 2009), indicating that aberrant MMP levels could give rise to abnormal spine morphologies. Consistent with this observation, active MMP-9 levels were found to be greater in hippocampal lysates of 1-week-old Fmr1-KO mice versus wild types (Bilousova et al. 2009). These data suggest that reducing MMP activity could help to normalize spine morphology. As minocycline and other tetracyclines are well known to inhibit the expression of MMPs [reviewed in (Griffin et al. 2010)], and mincycline treatment of Fmr1-KO pups reduced MMP-9 activity (Bilousova et al. 2009), it is likely that the spine effects described by Bilousova and colleagues could be due in part to the inhibition of an overactive enzymatic process.

Finally, Bilousova et al. (2009) also tested the effects of minocycline on several behavioral measures for the Fmr1-KOs; drug was given to nursing dams beginning at birth for 21 days and the pups were tested at 3 weeks of age. Using the elevated plus maze, the time spent in the open arm was used as an indicator of anxiety (less time = more anxious). Minocycline-treated Fmr1-KO mice spent more time in the maze's open arms as compared to nontreated mutants, indicating that the drug reduced their anxiety. Using the Y maze to examine hippocampal dependent memory, the investigators also found that minocycline treatment facilitated the Fmr1-KO's strategic exploratory behavior in the task. Overall, these findings indicate that minocycline could be a potential therapeutic for the treatment of cognitive impairment in FXS.

17.1.2.11 Human Trials of Minocycline—After publication of the Bilousova et al. (2009) report, numerous families began using minocycline in their children with FXS as a targeted treatment. Utari et al. (2010) surveyed 50 families who utilized minocycline in their children or adults with FXS for at least 2 weeks to 20 months (mean duration 3.5 mo). Seventy percent of individuals with FXS (43 males and 7 females mean age 13.3 years; SD 6.2 years) had a positive response to minocycline with improvements in language, behavior, and/or cognition as judged by the parents. Although this was not a controlled trial it suggested that further studies of minocycline are warranted for treatment of FXS. Paribello et al. (2010) reported positive effects in an open trial of minocycline in boys with FXS 13 years and older. Currently, a controlled trial of minocycline is taking place at the MIND Institute for children 3.5 years to 16 years with FXS. So far the trial has not demonstrated significant side effects and the analysis of efficacy will be carried out in 2011.

17.1.2.12 Antioxidants: Melatonin and Vitamin E—Results of studies using the Fmr1-KOs have suggested that antioxidants such as melatonin and vitamin E could be beneficial for aspects of the FXS phenotype. Melatonin is a hormone secreted by the pineal gland that acts to regulate the body's circadiam rhythm, in addition to other hormones. Melatonin is also a strong antioxidant and has been shown to be neuroprotective in animal studies (Singhal et al. 2010). Evidence for oxidative stress in the Fmr1-KO has been reported (el Bekay et al. 2007; Romero-Zerbo et al. 2009); for example, the mutants display reduced glutathione levels (in brain) and elevated lipid peroxidation (in brain and testes). Treatment with melatonin for 1 month normalized these biochemical measures in the mutant (Romero-Zerbo et al. 2009). In addition, measures of aberrant motor and learning behaviors as well as anxiety in the Fmr1-KOs also were normalized by melatonin treatment (Romero-Zerbo et al. 2009). Similarly, treatment of Fmr1-KOs with the strong antioxidant/free radical scavenger vitamin E (alpha-tocopherol) with or without *N*-acetyl L cysteine (NAC) has been shown to normalize oxidative stress markers, testicular size, and behavior (learning, anxiety) (de Diego-Otero et al. 2008). While these data suggest that melatonin and vitamin E could be useful for FXS, particularly as part of a combinational treatment strategy with another drug approach, for it is important to note that melatonin has a very short half-life. Consequently, synthetic melatonergic agonists may be more effective (Hardeland 2010).

17.1.2.13 Human Trials of Melatonin and Other Antioxidants—A controlled study of melatonin's effect on sleep has been carried out in six children with FXS, one with the premutation and in five children with ASD between the ages of 2 and 15.25 years (SD 3.6) (Wirojanan et al. 2009). This study lasted 4 weeks with a crossover design between melatonin and placebo. Children treated with melatonin demonstrated a significant increase in mean night sleep duration, a decrease in mean sleep onset latency, and an earlier sleep onset time compared to placebo. Dysregulated sleep occurs in 32–77% of patients with FXS (Richdale 2003; Kronk et al. 2010). However, these sleep problems are universal in many neurodevelopmental disorders including autism (Richdale 1999). Other controlled trials of melatonin have also been helpful for ASD (Garstang and Wallis 2006). It is also possible that the therapeutic effects of melatonin are related to its antioxidant effects and ability to normalize synaptic connections in the KO mouse (Romero-Zerbo et al. 2009). Although other antioxidants such as omega 3 fatty acid, vitamin C, vitamin E, and NAC have been utilized by many families often routinely there have been no controlled studies of their use. An exception to this is the controlled study of L acetylcarnitine (LAC) carried out by Torelli and colleagues (2008) in 63 males with FXS and ADHD treated for 1 year in a controlled parallel study of LAC (20–50 mg/kg/day up to 1,000 mg/day) versus placebo. Fifty six patients completed the study and there was a significant improvement in ADHD symptoms on the Conners Global Index Parents scale and also in behavior and socialization on the Vineland Composite and Socalization Scale with LAC compared to a placebo.

17.1.2.14 Human Trials of Donazepil and Other Agents—Other agents acting at an array of receptors have undergone exploratory study in groups with FXS [reviewed in (Berry-Kravis et al. 2011)]. These include donazepil, an anticholinesterase which raises acetyl choline in brain and is extensively utilized for maintenance of cognitive function in Alzheimer's disease. Donazepil showed promise for treatment of behavior and social

function in an open-label trial in participants with FXS and now is being studied in a larger placebo-controlled trial (clinicaltrials.gov). A small open-label study of memantine, an NMDA antagonist, in six individuals with FXS demonstrated modest clinical benefit on a CGI in 4/6 patients, but lack of improvement on behavioral rating scales, and several patients developed substantial irritability that limited treatment (Erickson et al. 2009). An open-label study of riluzole, a sodium channel blocker and glutamate uptake activator that indirectly decreases glutamate receptor activity, showed overall behavioral improvement in only one subject of five patients with FXS treated, although ERK activation rates normalized and there was a suggestion of improvement specifically in hyperactivity symptoms (Erickson et al. 2010c).

Anecdotal treatment experience with three adults with FXS treated with acamprosate, demonstrated improvement in language and behavior in all patients (Erickson et al. 2010a). Acamprosate is a drug approved for assisting with alcohol withdrawal that most likely interacts with multiple receptors but primarily may exert effects by acting as a mixed agonist/antagonist at NMDA receptors and activating GABA-A receptors with possibly inhibitory effects at group I mGluRs (Erickson et al. 2010a). One patient had significant gastrointestinal side effects that are often seen with acamprosate.

Cells from the *fmr1* knockout mouse and from individuals with fragile X show reduced cAMP production (Berry-Kravis et al. 1995; Kelley et al. 2007; Chen et al. 2010) which is dependent on FMRP levels (Berry-Kravis and Ciurlionis 1998). Likewise adenylate cyclase activity modulates mGluR-mediated regulation of FMRP activity (Wang et al. 2008a, b). Although the mechanism through which FMRP regulates cAMP production is not known, FMRP is known to bind adenylate cyclase subunit mRNAs (Darnell et al. 2011). Because of the reduction in cAMP production in FXS tissue, treatment with phosphodiesterase inhibitors such as rolipram has been proposed, however no preclinical work has yet been done in this area.

17.1.2.15 Genetic Manipulations—A few studies have attempted to correct the phenotype in the Fmr1-KO by genetic manipulation. The approach used with some success was either through the reduction of mGluR5 levels (see above) or by expressing FMRP de novo in the Fmr1-KO (Peier et al. 2000; Gantois et al. 2001; Musumeci et al. 2007; Zeier et al. 2009). Expression of FMRP in the Fmr1-KO rescued a number of the phenotypes including normalizing hippocampal LTD (Zeier et al. 2009), macroorchidism (Peier et al. 2000), reducing anxiety (Peier et al. 2000), and reducing audiogenic seizure susceptibility (Musumeci et al. 2007). While gene and/or stem cell therapy for neurological disorders is still in its early phase and has a number of issues, these studies are encouraging and suggest that this approach could correct the FXS phenotype (for comprehensive reviews see Chaps. 2 and 6).

17.1.2.16 Clinical Trial Design and Associated Hurdles in FXS—Although an ever-increasing number of neuronal targets for treating the underlying disorder in FXS are emerging, and have prompted early translational work, there are still many issues regarding optimal trial design and how to best demonstrate treatment effects in a clinical trial setting, in the absence of good models for cognitive treatment trials for any neurodevelopmental

disorders [reviewed in (Berry-Kravis et al. 2011)]. FXS, in fact, serves as a good model to develop such designs, particularly because FXS is a single genetic disorder in which all affected individuals have the same cellular defect as the primary cause of their brain disorder, a mouse model is available, information on the synaptic function of FMRP in brain is known, and aspects of FXS model more common disorders likely to have mechanistic overlap, including autistic spectrum disorders and learning disabilities.

Trial design issues that need to be resolved for each targeted treatment trial in FXS include: (1) length of placebo treatment and whether to use crossover designs or open-label extensions to ensure everyone gets a chance at active treatment and increased recruitment; (2) lack of information on optimal dosing and whether to determine this through dose escalation or flexible dosing within or between subjects or multiple fixed-dose arms; (3) how to best detect side effects in cognitively impaired individuals who may not be able to discuss their symptoms; (4) the most appropriate age range to study treatment effects, balancing concerns about safety that dictate studies in adult trials initially, with the possibility that studies at younger ages may be indicated even if there are minimal effects in older individuals, because much more significant results may be seen by treating the underlying disorder in young children who are not as advanced in the process of brain development, and are still in school; (5) understanding of the length of treatment needed to impact brain wiring and demonstrate measurable cognitive improvement; (6) drug formulation and how to best deliver drugs to younger children and individuals with difficulty swallowing pills due to coordination issues; (7) inclusion of females and mosaic individuals, and whether to analyze their responses separately, as individuals with FMRP present in a fraction of cells may have different dosing ranges, responses, and toxicities; (8) whether to allow baseline medications and the balance between the need to analyze treatment effects in the absence of medication interactions, problems with recruitment and patient deterioration if baseline medications have to be weaned, and the importance of demonstrating that new targeted treatments can actually improve symptoms even when the best available symptomatic regimen is already in place; (9) the numbers of study visits and travel issues for a relatively rare disorder in which subject numbers are limited and participants may travel a distance to get to a trial site; and (10) the problem of the lack of validated, sensitive behavioral outcome measures, the lack of well-defined cognitive outcome measures, and the lack of biomarkers known to correlate with functional status in FXS.

The design and validation of outcome measures for clinical trials in FXS and other neurodevelopmental disorders represents the most significant hurdle in trial design for targeted treatments. The choice of optimal outcome measures has been difficult because of the need to test a broad range of abilities so that there is not too much floor or ceiling effects with high- or low-functioning individuals, issues with co-operation and variable performance, the general lack of information on reproducibility of measures for the population being studied, and because for measures that would appear to quantify core defects, there is insufficient data available on whether they correlate with quality of life or true functional improvement. Only a subset of outcome measures utilized in recent trials have shown good feasibility and validity (Berry-Kravis et al. 2006, 2008a, b, 2009). Thus, recently investigators have begun to develop templates for pretrial feasibility,

reproducibility, and validity assessment (Berry-Kravis et al. 2008a, b; Hessl et al. 2008; Knox and Berry-Kravis 2009; Scaggs et al. 2011). The choice of outcome measures must also balance the use of standard accepted behavioral measures with precedent for use in drug registration/FDA approval, which are generally caregiver rating scales (such as the ABC), versus use of novel measures (Hessl et al. 2008; Knox and Berry-Kravis 2009; Scaggs et al. 2011) that are more quantitative and may objectively measure core phenotypes (such as eye tracking or PPI), thus advancing treatment science, but have no precedent for registration and are not yet known to predict a specific functional outcome.

A recent series of NIH-sponsored meetings (2009) aimed at developing a consensus about optimal outcome measures for clinical trials in FXS has resulted in some recommendations about best choice of currently existing measures, validation needs for existing measures, optimal additional measures that need to be developed, and the work needed to develop these. No one behavioral rating scale was felt to capture the range and character of problem behaviors typically observed in individuals with FXS, and the development and validation of a fragile X-specific behavioral scale has been suggested. As initial work in preparation for development of such a scale, ABC ratings have been collected from multiple sites and subjected to factor analysis for FXS relative to age and gender. This early work has indicated that the factor structure of ABC subscales and the number of items incorporated into the scale (which was developed for individuals with general cognitive impairment and has been used extensively in autism) should be modified for good validity in FXS (Hessl et al. 2010).

Several years ago the Fragile X Clinical and Research Consortium (FXCRC) was created to help ensure state-of-the-art care delivery to meet the needs of individuals with FXS in North America and facilitate large-scale research efforts and clinical trials. This organization is developing structure for collaboration of FXS clinics worldwide, in preparation for large multisite clinical trials that will be necessary for regulatory approval of targeted treatments.

17.1.2.17 Combining Targeted Treatments with Educational Interventions— Medications alone will not reverse the phenotype of those with FXS because the learning that has occurred throughout life has to be recovered also. The targeted treatments will improve synaptic connections but cognitive remediation needs to take place to strengthen these connections and remediate the lost learning unless treatment is started right after birth. This is pertinent to adults who are initiated into targeted treatments because they are out of school and not necessarily in a learning environment. Basic abilities such as reading or writing must be addressed in learning paradigms and often a tutor is an expense that cannot be afforded by many families. Therefore, computer learning programs can be utilized and targeted treatment studies should begin to address the efficacy of learning both with and without targeted treatments.

An example of a learning computer module is the Cogmed program developed by Torkel Klingberg (Cogmed Cognitive Medical Systems AB, Stockholm, Sweden) for the training of working memory. Working memory (WM), defined as the ability to temporarily store and manipulate information for some purpose (Baddeley 2000), is instrumental for a plethora of daily activities such as keeping track of goals and instructions and planning the next course

of action based on current conditions. From a psychometric standpoint, WM can be easily and objectively measured (e.g., through simple span tasks incorporating a simultaneous processing element, such as mentally reordering a series of digits/letters or pattern of blocks) with tasks that most people are capable of performing with varying degrees of success. It is therefore a natural target for cognitive intervention, despite the fact that WM was once thought to be a fixed and highly heritable construct independent of environmental influences (Miller 1956; Niaz and Logie 1993; Kremen et al. 2007; Engel et al. 2008). However, a wealth of recent research measuring the pre- and postintervention changes in response to a working memory training protocol support the plasticity of WM (Gunther et al. 2003; van't Hooft et al. 2003; Holmes et al. 2009; Klingberg 2009; Dahlin 2010; Holmes et al. 2010; Klingberg 2010). Furthermore, changes have also been measured on a neuroimaging level that show increased brain activity in parietal and frontal regions specific to WM after training (Olesen et al. 2004; Westerberg and Klingberg 2007) and on a chemical level through studies showing corresponding decreases in dopamine receptor D1 density as WM performance increases in brain areas known to be critical to WM performance (McNab et al. 2009). It has been shown that dopamine is an important neurotransmitter during WM tasks, a precise balance of which is necessary for optimal WM functioning such that too much or too little would be detrimental (Luciana et al. 1992; Müller et al. 1998; McNab et al. 2009).

Much has been learned from studies of populations with Attention Deficit/ Hyperactivity Disorder (ADHD) that can be applied to those with FXS with ADHD. WM is tied very closely to attentional processes, and neuroimaging studies even indicate activation of similar regions in the parietal and prefrontal lobes as controlled attention (Castellanos and Tannock 2002; Kane and Engle 2002; Olesen et al. 2004; Martinussen et al. 2005; Klingberg 2009; Beck et al. 2010). Moreover, individuals with ADHD typically present with deficits in WM function (Barkley 1996; Martinussen et al. 2005). Training in WM could therefore register improvements in cognitive domains, such as attention and focus, and the use of Cogmed in populations with ADHD has been well documented, with parents and teachers reporting reductions of symptoms postintervention (Klingberg et al. 2002, 2005; Beck et al. 2010).

A large percentage, ranging from 41 to 93% based on previous studies, of children with FXS also meet criteria from the Diagnostic and Statistical Manual for Mental Disorders (DSM IV) for ADHD subtypes (Bregman et al. 1988; Freund et al. 1993; Mazzocco et al. 1998; Backes et al. 2000). Furthermore, when compared to three other groups of intellectual disability, the FXS group exhibits a distinct attention impairment above and beyond that found in the other groups (Turk 1998; Munir et al. 2000; Cornish et al. 2004a), consistent with assertions that the fundamental neurocognitive deficit in FXS is in controlling the flow of input and (Cornish et al. 2004b; Mastergeorge et al. 2010), a function which is heavily reliant on WM. In addition, it has been hypothesized that intracellular levels of FMRP may rise in response to a WM load in typically developing individuals (Kwon et al. 2001), which would explain the abundant WM difficulties seen in individuals with FXS (Kwon et al. 2001; Lanfranchi et al. 2009; Baker et al. 2011). This corroborates studies on the Wechsler IQ scales which indicate that performance on the Digit Span subtest (a measure of WM) correlates with FMRP levels in males with FXS (Mastergeorge et al. 2010). This WM deficit is present even after controlling for overall IQ (Kwon et al. 2001), suggesting that there are disproportionately large WM impairments in FXS even in relation to global cognitive

impairment. All this is in line with previously described evidence of dopaminergic dysfunction in FXS (Hjalgrim et al. 1999) and the critical role dopaminergic transmission plays in WM function (Luciana et al. 1992; Müller et al. 1998; McNab et al. 2009; Klingberg 2010). Taken together, the fragile X population, with so many core deficits relating to WM and attentional dysfunction, is a prime candidate for Cogmed's working memory training.

Aside from the direct benefits that Cogmed can offer in the form of improvements in WM and the related domains of attention and focus, recent studies on WM also implicate a significant role in higher order cognitive functions such as learning, language/reading comprehension, and reasoning ability (Kyllonen and Christal 1990; Daneman and Merikle 1996; Fry and Hale 2000; Kane et al. 2005; Dahlin 2010), all of which require holding and consistently reevaluating information in WM in the midst of new incoming information. Additionally, WM capacity has also been shown to be an effective longitudinal predictor of academic achievement (Gathercole et al. 2003; Biederman et al. 2004; Alloway and Alloway 2010), correlating most strongly with abilities in reading (Gathercole and Pickering 2000; Swanson and Sachse-Lee 2001b) and arithmetic (Swanson and Sachse-Lee 2001a; Geary et al. 2004). Furthermore, other studies indicate a positive correlation between performance on tasks measuring WM and tasks measuring fluid intelligence, or the ability to reason out novel tasks independent of previously acquired knowledge (Gray et al. 2003; Jaeggi et al. 2008). More broadly, WM tasks have also been shown to correlate with Charles Spearman's *g* (Suβ et al. 2002; Conway et al. 2003), a statistical variable describing the theoretical general reasoning ability that underlies the shared variance between all cognitive tests (Spearman 1927). In other words, people who perform well on one cognitive test generally perform well on others, and the common denominator that promotes this correlation is termed "*g,*" of which WM is thought to be the most important deriving factor (Suβ et al. 2002). Therefore, training in working memory can have far-reaching effects beyond merely the realm of ADHD; it can also positively influence general intellectual functioning in many different domains of life. Since FXS is the most common known cause of inherited intellectual disability (ID) (IQ < 70; Chonchaiya et al. 2009), WM cognitive training is a natural intervention strategy to explore. Although to date no compelling evidence exists of which the authors are aware that overall IQ scores are affected by WM training, studies of this nature tend to involve IQ testing within a short time frame after training (i.e., Holmes et al. 2010), which may not allow enough time for the full remediating effects of WM training on learning and reasoning abilities to take effect. It's possible that given a longer time frame in the course of years after training, WM training may create a positive feedback loop in which these individuals are more mentally engaged in all aspects of their daily lives and are therefore more prone to further beneficial environmental stimulation (thereby continuously maintaining their WM training), such that performance on IQ tests may improve significantly. Additionally, it's possible that although the effects of WM training on IQ may not be readily apparent in populations such as ADHD, it may be more so in a population such as FXS, where the initial deficits are more pronounced and the lifetime intellectual trajectory has a downward trend relative to the general population, thereby creating more room to detect improvement. This could be especially evident when WM training is used in conjunction with partial correction of synaptic dysfunction by a

targeted treatment regimen, such that new cognitive development, not possible with training alone, can now be achieved.

In summary, the current and future aspects of intervention for FXS are promising for improving not only the behavioral aspects but also the cognitive aspects of this syndrome. Future FXS treatment may be a portal for understanding targeted treatments in a variety of neurodevelopmental disorders including autism.

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Table 17.1

FXS targeted treatments in models and man

	Phenotypes reversed		Translational progress
Agent/Target	*dfmr* mutant Fly	*Fmr1* k/o Mouse	Humans with FXS
(1a) Block translational signaling pathway-external			
mGluR5 inhibition (MPEP, fenobam, STX107, AFQ056, RO49917523); MPEP used in models see footnote (a)	Courtship behavior – immediate recall and short-term memory; Mushroom body formation; Odor-shock memory; Survival on glutamate-containing food	Audiogenic seizures[a]; Epileptiform bursts; Open field hyperactivity; Dendritic spine morphology[a]; Amygdala mEPSP frequency; Prepulse inhibition[a]; Marble burying[a]	Fenobam – phase IIa single-dose, open-label trial – PPI improved, anxiety reduced; AFQ056 – phase II[b] trial completed, phase III trial being initiated; RO4917523 – phase II trial in progress STX107 – phase I completed
mGluR5 inhibition by genetic reduction of mGluR5 receptors		Audiogenic seizures; Dendritic spine density; Excessive protein synthesis; Abnormal growth pattern; Ocular dominance plasticity; Inhibitory avoidance extinction	
(1b) Block translational signaling pathway – internal			
Lithium (inhibition of GSK3β and PI turnover)	Courtship behavior – immediate recall and short-term memory; Mushroom body formation	Audiogenic seizures; Open field hyperactivity; Dendritic spine morphology; Learning and anxiety deficits in the elevated-plus maze, elevated zero maze, passive avoidance; Social interaction deficit with new mice and anxiety-related behaviors during social interaction	Open-label trial – behavioral improvement, some adaptive skills and verbal memory improved; ERK biomarker normalized
GSK3β inhibition (AR-A014418 or SB-216763)		Audiogenic seizures	
PAK inhibition by genetic reduction of PAK		Dendirtic spine morphology; Cortical LTP deficits; Open field hyperactivity, repetitive behaviors, center field anxiety deficit; Fear conditioning	
PI3K inhibition (LY294002)		Dendritic spine morphology; mTOR overactivity	
ERK / MEK inhibition (SL327)		Audiogenic seizures; Protein synthesis	
(2) Inhibit activity of individual FMRP-regulated proteins			
Inhibit MMP9 (minocycline)		Dendritic spine morphology; Anxiety in elevated plus maze; Exploratory behavior in Y maze	Improvement in behavior in small open- label trial

Results Probl Cell Differ. Author manuscript; available in PMC 2014 July 29.

	Phenotypes reversed		Translational progress
Inhibit APP/Aβ with antibody or by genetic reduction of APP		Audiogenic seizures; Dendritic spine morphology; Marble burying	
Inhibit STEP by genetic reduction of STEP		AMPA receptor internalization; Audiogenic seizures; Open field hyperactivity	
(3) Activate surface AMPA receptors			
Ampakines (CX516, CX614)		CX614 increases BDNF which reverses impairments in hippocampal TBS-LTP	CX516 – phase II trial – no cognitive or behavioral effects overall – dose too low but may have helped subjects co-treated with antipsychotics
(4) Other synaptic receptors/proteins			
GABA-B agonists (baclofen, R-baclofen)	Survival on glutamate-containing food; Memory deficits	Audiogenic seizures; Open field hyperactivity; Marble burying	R-baclofen phase II trial – improvement in overall function, social and language function in more socially impaired subject group
GABA-A agonists (ganaxolone)		Audiogenic seizures	
Anticholinesterase (donazepil)			Open-label trial – behavioral and social improvement
NMDA antagonists (memantine, acamprosate)			Memantine – small open-label trial – no overall improvement Acamprosate – open-label trial in three patients with improved language and socialization
Glutamate uptake inhibition (riluzole)			Riluzole – small open-label trial – no overall improvement, ERK biomarker normalized

[a] Audiogenic seizures – MPEP, fenobam and STX107; Spine shape – MPEP, fenobam, AFQ056; PPI – MPEP, fenobam, AFQ056; Motor learning – MPEP, fenobam, AFQ056; Marble burying – MPEP, fenobam, STX107

[b] All phase II or III trials listed in the table are placebo-controlled double-blind trials unless otherwise noted. Adapted from Berry-Kravis and Knox (2011)