Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Targeted therapies for fragile X syndrome: current state and future direction of clinical trials in humans

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Fragile X syndrome (FXS) is the most common monogenetic and testable cause of intellectual disability and autism spectrum disorders. FXS is caused by a 'full mutation' (>200 CGG repeats) in the *FMR1* gene. The phenotypic features of FXS are highly variable, frequently include autistic features or other behavioral abnormalities, and may comprise a distinctive appearance. In recent years, considerable progress has been reached in our understanding of the molecular pathophysiology and the impairment of neurobiological processes underlying the manifestation of FXS, to a large extent derived from work on animal models, especially on *FMR1*-knockout mice. These discoveries have led to the initiation of clinical trials with novel targeted drug treatments in humans with FXS. This review provides an overview of selected promising therapeutic targets and the current state of these clinical trials.

Keywords: autism spectrum disorders • *FMR1* • FMRP • fragile X syndrome • metabotropic glutamate receptors • protein synthesis • signal transduction • synaptic plasticity • targeted treatment

Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability, learning disability and of the 'syndromic' forms of autism spectrum disorders (ASD), that is, disorders due to mutation of a single gene where ASD-associated behavior commonly occurs. The mutation causing FXS is found in 1–6% of boys with ASD [1]. Other genes involved in 'syndromic' forms of ASD include *MeCP2*, responsible for Rett syndrome (RTT), and *TSC1/2*, causing the tuberous sclerosis complex (TSC; reviewed in [2]). Within the past 10 years, various animal models of these single-gene disorders have yielded a wealth of valuable information regarding the understanding of the altered molecular neurobiology and pathophysiology, biochemistry and morphology and therefore of the causes of the characteristic behavioral and cognitive features of these disorders on a molecular level. It is increasingly recognized that the molecular pathways involved in these disorders considerably overlap. For various reasons, FXS is particularly well suited as a model example of a neurodevelopmental disorder for the translation of findings of basic neuroscience research to molecularly targeted treatments:

- Multiple animal-model systems with a well-characterized phenotype are available, which have provided important insights into the molecular mechanisms causing altered synaptic plasticity and morphology and ample opportunities to test therapeutic options in a preclinial phase (reviewed in [3]);
- The core phenotype of FXS predominantly includes features of other more common 'idiopathic' neurodevelopmental disorders, such as learning disability and attention deficit hyperactivity disorder, with approximately 30% of boys [1] and 20% of girls [4] fulfilling diagnostic criteria for autism;

FUTURE SCIENCE part of fsg

- Although FXS is generally a rare disease, due to its relatively high prevalence (as compared with other rare monogenetic disorders) a sufficient number of patients can potentially be recruited for clinical trials.

However, clinical trials in FXS patients pose a considerable number of specific challenges that need to be addressed: first, although FXS is a single-gene disorder, there is high phenotypic variability within a broad spectrum of potential symptoms including cognitive, behavioral and morphological features with, for example, the degree of cognitive impairment ranging from mild cognitive impairment to severe intellectual disability. This variability impedes the possibilities for defining and appropriately measuring primary end points for clinical studies and may result in a need to predefine clinically distinct subgroups of patients.

Second, key questions that need to be resolved are the best time point/age range to initiate treatment and the length of treatment required. It is currently unclear whether there is a 'window of opportunity' for successful therapeutic interventions depending on the potential reversibility of the functional and morphological changes observed in FXS as a typical neurodevelopmental disorder. This necessity needs to be weighed against safety concerns regarding treatment in young children, whose CNS are still in the process of maturation and who may therefore on the one hand have a higher probability to benefit from early treatment, but on the other hand may also have a higher vulnerability to potential side effects. The fact that a diagnosis of FXS, like many other neurodevelopmental disorders, is frequently not established unambiguously until early childhood development is completed, emphasizes the importance of these questions.

Third, the complex and sometimes severe phenotype of FXS results in several practical problems for clinical studies, such as how to deliver oral medication to younger children or patients with orofacial dyspraxia, or how to detect possible side effects in patients with severe intellectual disability, who may not be able to adequately report them, and so on. These particular features of FXS, some of which are shared with other neurodevelopmental disorders, may be reasons as to why many of the clinical trials that have been conducted to date are small, open-label studies in a limited number of patients, the results of which are sometimes more difficult to interpret than larger, placebo-controlled, double-blind studies.

Genetics & phenotypes of *FMR1*-related disorders

Genetics of FXS & fragile X-associated tremor/ataxia syndrome

In 1943, James Purdon Martin and Julia Bell first clinically described FXS, originally known as Martin–Bell Syndrome, in a family with eleven affected males as a disorder of inherited cognitive impairment with X-chromosomal inheritance [5]. Twenty-six years later, a fragile site at the distal end of the long arm of the X chromosome (Xq27.3) was observed cytologically in FXS patients [6,7]. In 1991, the genetic defect underlying FXS was finally revealed by mapping this fragile site to a specific chromosomal location and demonstrating that it contained an expanded trinucleotide (CGG) repeat in the 5′ untranslated/promoter region of a novel gene that was named fragile X mental retardation 1 (*FMR1*) [8]. Expansion of this CGG repeat to more than 200 repeats (full mutation) was found to cause hypermethylation of the promoter region, which leads to transcriptional silencing of *FMR1,* eventually resulting in typical symptoms of FXS. In addition, further epigenetic changes with deacetylation of histones H3 and H4, reduced methylation of lysine 4 (K4) and an increase in methylation of lysine 9 (K9) have been demonstrated in FXS patients [9,10]. The protein encoded by *FMR1*, FMRP, was characterized in 1993 [11] and found to be an RNA-binding protein with diverse functions expressed in most cell types with particularly high levels in the brain and testes. FMRP was shown to play an important role in the regulation of protein synthesis at the dendrite in response to neural activation under certain conditions. In contrast to the full mutation, which causes FXS manifesting in childhood, smaller CGG repeat expansions (between 55 and 200 repeats), which are termed premutation, may be associated with a phenotypically very different condition manifesting in late adulthood, fragile X tremor/ataxia syndrome (FXTAS; reviewed in [12]), and/or with fragile X-associated premature ovarian insufficiency. Data obtained from animal models suggest that FXTAS, in contrast to FXS, is due to a toxic mRNA gain-of-function mechanism mediated by elevated levels of CGG-repeats containing *FMR1*-mRNA, causing neuronal toxicity and neurodegeneration. Infrequently, in individuals with large premutation alleles (150–200 repeats), some features of FXS such as mild cognitive deficits may occur, presumably due to lowered levels of FMRP [13]. In general, girls with FXS are more variably and less severely affected than boys due to the presence of a normal X chromosome and depending on the ratio of inactivation of the nonmutated *FMR1* allele and resulting levels of FMRP. Although much more frequent in males with the premutation, FXTAS may also occur in females, apparently with a different phenotypic spectrum (reviewed in [14]).

Phenotype of FXS & FXTAS

In addition to mild-to-moderate cognitive deficits, boys with the full mutation frequently suffer from behavioral problems such as hyperactivity, autistic features with

deficits in social interaction (e.g., poor eye contact and shyness), repetitive behavior (hand biting and hand flapping) and repetitive language, attention deficits, hypersensitivity to sensory stimuli, anxiety and mood lability. Typical physical features include an elongated face with prominent ears, a high arched palate, macroorchidism, hyperextensible joints, soft skin and flat feet. Other medical problems comprise epileptic seizures, mitral valve prolaps and frequent ear infections. Seizures are observed in approximately 13–18% of boys and 5% of girls, frequently as complex–partial seizures that generally respond well to anticonvulsive medication and, in the majority of patients, spontaneously resolve during childhood [15]. Aggression may occur in up to 30% of males [16], most frequently in adolescence and often only as a temporary problem. In contrast, girls with FXS generally show less severe cognitive deficits, typically manifesting as learning disabilities, and milder behavioral abnormalities that may include impulsivity, attention deficits, shyness, social anxiety, specific phobias, selective mutism and executive function deficits [16,17]. In contrast to FXS, FXTAS typically occurs in older male carriers of the premutation and is characterized by a slowly progressive combination of symptoms including a mixed tremor (intention, postural and rest) with prominent intention tremor and gait ataxia and, more variably, dementia with executive dysfunction, features of parkinsonism, polyneuropathy and autonomic deficits (reviewed in [12]).

Function of the FMRP protein & functional & morphological consequences of its loss in FXS: the mGluR theory of FXS

Early biochemical studies on FMRP demonstrated its association with translating polyribosomes and implied a role for FMRP in the regulation of protein synthesis at synapses (reviewed in [18]). Subsequent studies revealed that FMRP is a protein binding mRNAs with G-quartet (Gq) motifs, which is found in the cytoplasm of many cell types but most abundantly in neurons. FMRP regulates the translation of a large variety of mRNAs in the brain (~4% of total brain mRNA) in response to neural activation by acting as a 'translational brake' on the synthesis of a subset of both pre- and post-synaptic/dendritic proteins [19]. In addition, FMRP appears to be involved in the transport and localization of these mRNAs to dendrites and synapses [20,21]. Consistent with a role for FMRP in synaptic maturation and plasticity, an altered morphology of dendritic spines with an immature, abnormally long and tortuous appearance, which is normally found only in early stages of neocortical development, and an increase in their density has been observed in post-mortem brain tissue of both FXS patients and *FMR1*-knockout mice [22–24]. These alterations appear to be due to a deficit in 'pruning' of unneeded synaptic connections, a process important in the regulation of physiologic synaptic development, which is apparently at least partly mediated by FMRP. Increasing evidence from *FMR1*-knockout animal models, especially knockout mice, suggest that loss of FMRP leads to deficits in synaptic plasticity, for example exaggerated long-term depression (LTD) in the hippocampus and the cerebellum [25,26] and alterations/deficits in long-term potentiation in the cortex and hippocampus (e.g., [27,28]). In parallel, research on metabotropic glutamate receptors (mGluRs) over many years revealed an important role for mGluRs in activity-dependent synaptic plasticity (reviewed in [29]). Findings in *FMR1*-knockout mice suggested that synthesis of FMRP in response to activation of group I mGluRs (mGluR1/mGluR5) [30] leads to repression of the translation of other synaptic proteins involved, for example, in LTD, including MAP1B, PSD95, CaMKII, STEP, PIKE, APP, Arc, PP2A, potassium channel Kv3.1b and others; in the absence of FMRP, there is excessive basal translation and upregulation of these synaptic proteins [25,31,32]. Eventually, these converging lines of research led Bear and colleagues to propose the mGluR theory of FXS [33], which suggests that the deficits associated with FXS, due to loss-of-function of FMRP, are caused by upregulation of proteins synthesized in response to mGluR1/5 activation, and that treatment with mGluR1/5 antagonists in the absence of FMRP might be able to reverse some of these deficits by restoring normal levels of synthesis of these synaptic proteins, suggesting mGluR1/5 antagonists as a potentially suitable targeted treatment for FXS (Figure 1). One important mechanism mediating mGluR-dependent LTD is internalization of AMPA receptors; consistent with the mGluR theory of FXS, an increased loss of surface AMPA receptors is observed in *FMR1*-knockout mice [26,34].

However, it should be noted that, in addition to mGluRs, FMRP appears to influence translational pathways activated by other classes of receptors involved in neurotransmission, including muscarinic (M1) acetylcholine receptors [35] and dopamine D1 receptors [36,37]. Furthermore, the GABAergic system, which is a major inhibitory neurotransmitter system in the brain, has also been proposed to play an important role in the pathogenesis of FXS. This is supported by decreased levels of various GABA-A receptor subunits in *FMR1*-knockout animal models [38–40] and by direct binding of FMRP to the mRNA of the δ-subunit of the GABA-A receptor [21]. It is also important to bear in mind that only a fraction of the large variety of mRNAs that are bound by FMRP in the brain are directly associated with mGluR5 signaling [19].

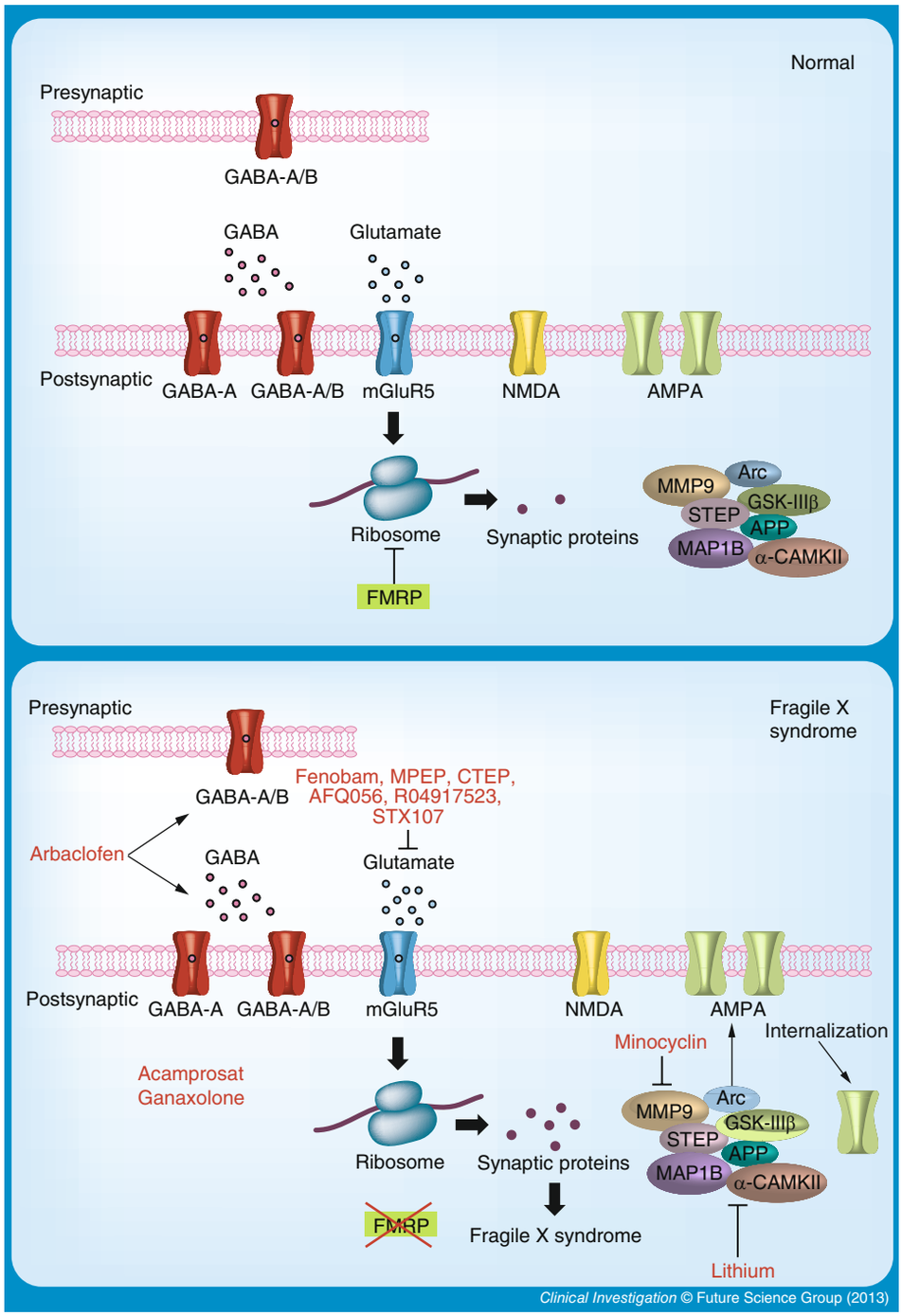


Figure 1. FMRP is a negative modulator of mGluR5-dependent synthesis of synaptic proteins/molecular pathways involved in fragile X syndrome. Under physiologic conditions, mGluR5 activation triggers synthesis of a set of synaptic proteins involved in synaptic plasticity, which is negatively regulated by the FMRP protein. Absence of FMRP protein in animal models and in fragile X syndrome (FXS) patients leads to excessive synthesis of these proteins and eventually to symptoms of FXS. The mode of action, according to current knowledge, of various compounds currently used in clinical trials in patients with FXS is depicted.
Modified from [91] and [92].

Potential targets for therapeutic interventions

The abovementioned findings on the mode of action of FMRP as a translational repressor regulating synthesis of synaptic proteins normally induced by activation of mGluR and other Gq-coupled receptors have spurred enormous interest in target molecules for possible treatment options of FXS in humans, which have been tested in established *FMR1*-knockout animal models. The aims of these treatments at a molecular level can be classified into the following groups:

- Decreasing activity in pathways induced by activation of group I mGluRs or other synaptic Gq-coupled receptors, either at the extracellular level (using mGluR5-receptor antagonists; e.g., fenobam) or at the intracellular level (using proteins inhibiting signal transduction pathways; e.g., lithium);
- Increasing the expression and activity of surface AMPA receptors (e.g., ampakines);
- Decreasing the activity of individual synaptic proteins, the synthesis of which are regulated by FMRP, either at the protein level (e.g., minocyclin as an inhibitor of MMP9) or at the mRNA level using antisense oligonucleotides;
- Modifying the activity of other synaptic proteins or receptors (e.g., GABA-A/B agonists or NMDA antagonists);
- Restoring *FMR1* gene activity by epigenetic modulators targeting potentially reversible epigenetic changes, primarily DNA methylation (e.g., 5-azadC).

Currently, the most extensive data from clinical studies in humans are available for drugs decreasing activity in pathways induced by activation of group I mGluRs, especially mGluR5 inhibitors, whereas mostly only small, open-label trials have

been completed for substances of the other groups. A schematic of the known or hypothesized mode of action of some of these compounds, according to current knowledge, is given in Figure 1.

Preclinical work

Glutamatergic system

In *FMR1*-knockout mice, a 50% reduction of mGluR5 protein levels by genetic engineering leads to a correction or prevention of most aspects of the FXS phenotype [32]. These genetic experiments served as a proof-of-principle to confirm the mGluR theory of FXS. In a *Drosophila melanogaster* model of FXS based on the loss of *dFMR1* activity, treatment with MPEP, a potent negative allosteric modulator of mGluR5 receptors that crosses the blood–brain barrier, resulted in rescue of most phenotypic aspects including restoration of synaptic plasticity, courtship behavior, and mushroom body defects [41]. Likewise, treatment of *FMR1*-knockout mice with MPEP was able to reverse a number of FXS-related phenotypes including epileptiform discharges in hippocampal slices and increased density of dendritic filopodia [42–44]. However, although fenobam and MPEP have long been known as effective and specific mGluR5-receptor antagonists, one major drawback is that they are extremely short-acting. Furthermore, one important question concerning clinical studies in patients with FXS is the optimal time point to initiate therapy, since the symptoms and deficits associated with FXS have long been assumed to be determined within a critical period of CNS development in early childhood before the age of 3 years. Importantly, it has recently been demonstrated that treatment of *FMR1*-knockout mice with CTEP, a long-acting mGluR5 inhibitor that can be administered orally [45], is able to at least partially reverse most FXS-associated phenotypic features, even if treatment is not initiated until young adulthood (mice aged 4–5 weeks) [46] . Whereas acute CTEP treatment corrected elevated protein synthesis in hippocampal slices, LTD, and susceptibility to audiogenic seizures, chronic treatment of *FMR1*-knockout mice with this drug (up to 17 weeks) improved several cognitive deficits and hypersensitivity to sensory stimuli, reduced the increased density of dendritic spines in the visual cortex and overactive ERK and mTOR signaling, and partially improved macro-orchidism. Taken together, these results support the notion that these symptoms are not irreversibly determined during early embryonic and/or postnatal development, but are at least partially due to ongoing processes of synaptic plasticity, and therefore accessible to pharmacological treatment even after brain maturation is largely complete, raising hopes for clinical studies with targeted treatments in FXS patients. Chronic treatment of *FMR1*-knockout mice with another mGluR5 receptor antagonist, AFQ056/mavoglurant (Novartis; Basel, Switzerland; see below), was recently shown to restore sociability behavior in a three-chambered task [47]. Further hints that modification of the FXS phenotype may be possible even in adulthood comes from a study in FMRP-conditional knockout mice, which suggests that deficits in adult neurogenesis may contribute to learning impairments in FXS patients, which in a mouse model can apparently be improved by restoration of FMRP expression specifically in adult neural stem and progenitor cells [48].

GABAergic system

First hints for an involvement of the GABAergic system in FXS came from studies in *FMR1*-knockout mice and in *dFMR*-mutant flies, which demonstrated decreased expression of the GABA-A receptor δ-subunit and of seven additional GABA subunits in *FMR1*-knockout mice [39] and decreased expression of the three GABA-receptor subunits in FMRP-deficient *D. melanogaster*, presumably at least partially due to destabilization of the mRNAs encoding these proteins [49,50]. Moreover, treatment with a GABA agonist, gabodaxol, was shown to rescue the neuronal hyperexcitability observed in primary neurons from the amygdala of *FMR1*-knockout mice [51]. More recently, treatment of *FMR1*-knockout mice with the selective GABA-B receptor agonist arbaclofen, the R-enantiomer of the well-known drug baclofen with apparently higher potency, has been demonstrated to rescue several important aspects of the FXS phenotype [52]. These results support the notion that arbaclofen may be a promising new therapeutic approach to treat FXS in humans. Furthermore, treatment of *FMR1*-knockout mice with the GABA-A receptor agonist ganaxolone was shown to reduce audiogenic seizures [53].

Other target proteins/substances: MMP9/minocyclin/lithium

MMP9 is one of many synaptic proteins for which the mRNA is a cargo for FMRP and for which the translation levels have been shown to be increased in the absence of FMRP; for example, in *FMR1*-knockout mice. Minocyclin is an antibiotic that inhibits MMP-9 and has recently been tested as a treatment option in stroke and various neurodegenerative diseases, mainly based on its antiapoptotic properties, as well as in autism. Treatment of young *FMR1*-knockout mice with minocyclin has been shown to promote the maturation of dendritic spines in the hippocampus, as well as in cultured hippocampal neurons of these mice, and to improve behavioral symptoms [54].

In contrast, lithium is an example for a well-known substance that is commonly used for the treatment of bipolar disorder, but that is also known to inhibit several

proteins involved in regulating translation of various proteins downstream of FMRP, partly in response to mGluR activation; for example, by inhibiting the PLC signaling pathway and GSK-3β, which are overactive in FXS animal models. Treatment with lithium has been tested in various animal models of FXS, resulting in amelioration of several phenotypic aspects of FXS in these model systems, including *dfxr* mutant flies [41] and *FMR1*-knockout mice [55–57]. These results suggest lithium as another suitable candidate for the treatment of FXS in humans.

■ Epigenetic modulators

This therapeutic approach is based on two observations: the open reading frame of the *FMR1* gene is intact in patients with FXS; and, rare cases of individuals with normal intelligence carrying fully or partially unmethylated *FMR1* full mutation alleles (>200 CGG repeats) have been reported (e.g., [58,59]), suggesting that restoration of *FMR1* gene activity may be possible by reversing the abovementioned epigenetic changes, that is, mainly DNA methylation of most cytosines of the CGG stretch and of the upstream sequence. *In vitro*, restoration of mutant *FMR1* gene activity by treatment with 5-azadeoxycytidine (5-azadC) on the mRNA and protein level in lymphoblastoid cells of FXS patients has been demonstrated [60,61]. However, hope that this approach may also be feasible *in vivo* has been dampened by safety issues concerning treatment with 5-azadC in humans, by concerns that unintended demethylation of other genes may occur and by the fact that it is presumably only active in dividing cells (excluding neurons, which are nondividing). Currently, no other safe demethylation drugs that are able to effectively reactivate the mutant *FMR1* gene have been reported.

Current state of clinical trials with targeted treatment in patients with FXS

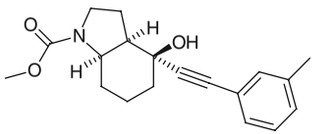
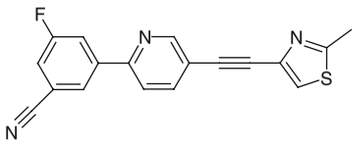
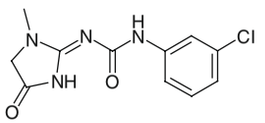
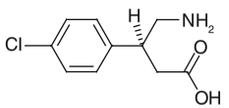
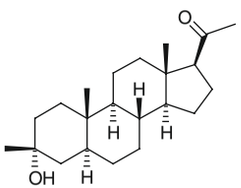
■ mGluR-receptor antagonists/glutamatergic system

The abovementioned highly encouraging results from studies of mGluR-receptor antagonists in FMRP-deficient animal models, primarily *FMR1*-knockout mice, raised hopes that these substances might be able to at least partially reverse the symptoms associated with FXS in humans. Historically, the imidazol derivative fenobam was the first mGluR-receptor antagonist to be implemented for clinical studies. This drug was originally developed in the 1970s for the treatment of anxiety disorders, with a reasonable safety profile but only moderate effectiveness [62]. At that time, its molecular target was unknown; it was not until 2005 that fenobam was demonstrated to be a selective mGluR5 antagonist [63]. Four years later, results of a small, single-dose open-label pilot study in adult patients with FXS were published [64]. Some modest beneficial effects were observed, with a rapid reduction in anxiety and hyperarousal and an improvement of prepulse inhibition in approximately 50% of patients, without relevant safety concerns. However, the pharmacokinetic properties of this substance with variable serum concentrations appeared problematic. Therefore, subsequent and current clinical trials focused on other mGluR-receptor antagonists with a more suitable pharmacokinetic profile (Table 1). One of these substances, R04917523, also known as RG7090 (Hoffmann-La Roche; Basel, Switzerland), which was originally developed for the treatment of depression, is currently in clinical development. Since early 2012, large multicentric, international, placebo-controlled, double-blind, Phase IIb studies with this compound are ongoing (Table 1) [101,102], with 180 probands with FXS aged 14–50 years treated over 12 weeks with either placebo, 0.5 or 1.5 mg of R04917523. First results are expected in the fourth quarter of 2013. Phase IIa studies were completed in September 2011, but results have not yet been published. Further clinical studies with R04917523/RG7090 are planned, both placebo-controlled and open-label, follow-up studies for 2 years, also in children and adolescents with FXS.

Another mGluR5-receptor antagonist with a more favorable pharmacokinetic profile when compared with fenobam, is AFQ056/mavoglurant. This drug was originally developed for the treatment of levodopa-induced dyskinesias in patients with Parkinson's disease, with promising preliminary results for this indication [65]. A small, short-term, double-blind, placebo-controlled, two-period crossover study in 30 adult male FXS patients treated with AFQ056 over 28 days showed significant improvement of the primary outcome measure, the Aberrant Behavior Checklist-Community Edition (ABC-C) score, after 19 or 20 days of treatment without causing serious side effects. Interestingly, however, this effect was observed only in a subset of seven patients, that is, those with full *FMR1*-promotor methylation and therefore transcriptional silencing of the gene with no detectable *FMR1* mRNA [66]. Currently, large multicenter and international Phase II and III clinical trials with AFQ056 in adult and adolescent patients with FXS are underway (Table 1) [103–106], with open-label, follow-up studies scheduled for at least 2 years. Further clinical studies, also in children with FXS, are planned. So far, no serious safety concerns have been reported in any of these studies.

A third mGluR5-receptor antagonist, STX107 (Seaside Therapeutics; MA, USA), is currently in clinical development (Table 1). One single-dose, Phase I study in healthy adult volunteers has been completed [107], whereas recruitment for a small, randomized, double-blind, placebo-controlled Phase II study with 16 adult subjects is currently suspended [108].

Table 1. Ongoing or recently finished clinical studies with mGluR5-receptor antagonists and drugs targeting the GABAergic system.

Substance/ code	Company	Structure	Phase	Dosing (mg/d)	Other indications	Remark	ClinicalTrials Database identifiers (subject ages)	Status (as of June 2013)	Ref.
RO4917523/ RG7090	Hoffmann-La Roche	NP	II	1 × 0.5–1.5	Depression	–	NCT01517698 (adolescents and adults) NCT01015430 (adults)	Recruiting Completed	[101,102]
AFQ056/ Mavoglurant	Novartis		II/III	2 × 25–100	LID-PD, HD, others	–	NCT01253629 (adults) NCT01357239 (adolescents) NCT01348087 (adults) NCT00718341 (adults)	Recruiting Recruiting Recruiting Completed	[103–106]
STX 107	Seaside Therapeutics		I	NP	None	–	NCT00965432 NCT01325740 (adults)	Completed Suspended	[107,108]
Fenobam	Neuropham		I	100†	None	Development stopped	–	–	
Arbaclofen	Seaside Therapeutics		II/III	2 × 5–3 × 10	ASD, spasticity, others	–	NCT01282268 (adolescents and adults) NCT01325220 (children) NCT00788073 (adolescents and adults)	Active, not yet recruiting Active, not yet recruiting Completed	[109–111]
Ganaxolone	Marinus Pharmaceuticals		II	3–12 mg/kg, max 1500	PTSD, infantile spasms, epilepsy	–	NCT01725152 (children and adolescents)	Recruiting	[112]

†Highest dose tested in one single-dose study.

ASD: Autism spectrum disorders; HD: Huntington's disease; LID-PD: Levodopa-induced dyskinesias in Parkinson's disease; NP: Not published; PTSD: Post-traumatic stress disorder.

Arbaclofen/GABAergic system

Recently published results of a Phase II clinical trial with arbaclofen (STX209) in 63 patients (children, adolescents and adults) with FXS, showed that the drug was well tolerated, with sedation and headaches as infrequent side effects. Although no difference from placebo was observed in the primary outcome measure, the Aberrant Behavior Checklist (ABC)-Irritability subscale, *post hoc* analysis revealed treatment benefit as measured by the ABC social avoidance scale, a newly validated scale specifically developed for the assessment of behavioral problems of patients with FXS [67]. This benefit was more pronounced in a subgroup of 27 patients with elevated social avoidance scores. Taken together, the results of this exploratory Phase II study suggest that arbaclofen and/or other GABA (B)-receptor agonists may improve behavioral problems and social interaction of FXS patients. Phase III trials with arbaclofen are currently ongoing (~120 patients; Table 1) [109–111], and the results of the abovementioned Phase II study have been taken into account to redefine the primary outcome parameter for this Phase III study.

In addition, a large Phase II study with a GABA-A receptor agonist, ganaxolone, in children and adolescents with FXS, is currently ongoing (Table 1) [112]. This compound is also being investigated for its anticonvulsant effects. Furthermore, a previous small open-label study with acamprosate in three patients with FXS over 21 weeks showed global clinical benefit with improvement of communication [68]. Acamprosate has both GABAergic and NMDA-antagonistic and possibly also mGluR-inhibitory properties, and is commonly used for the treatment of alcohol withdrawal syndrome. A Phase III study with acamprosate in children and adolescents with FXS (ages 5–17 years) over 10 weeks is currently ongoing [113].

Other substances: MMP9/minocyclin/lithium

The abovementioned results from preclinical studies in *FMR1*-knockout animal models increased interest in minocycline (which is frequently used for treatment of acne in adolescence) as a potential treatment option in FXS. A retrospective survey study of 50 children and adults with FXS who had been treated with minocycline for at least 2 weeks up to several months (mean 3.5 months) using a questionnaire, revealed gastrointestinal problems as a frequent side effect and discoloration of nails in one patient; parents reported improvement of language, attention, social communication and anxiety [69]. Furthermore, a small open-label add-on pilot trial in 20 patients with FXS (aged 13–32 years) over 8 weeks, demonstrated significant improvement of behavioral problems as measured by the ABC-C irritability subscale as the primary end point ($p < 0.001$) and in five out of six secondary end points. Treatment was generally well tolerated, with minor diarrhea and seroconversion to positive antinuclear antibodies as side effects [70]. These preliminary results led to a larger single-center, double-blind, placebo-controlled crossover clinical trial with minocycline in children with FXS (3.5–16 years of age) over two 3-month periods [71]. This study showed moderate, but statistically significant improvement for minocycline compared with placebo in one primary outcome parameter, the Clinical Global Impression Scale (2.97 ± 0.13 vs 2.49 ± 0.13, respectively; $p = 0.0173$), without serious adverse events. However, results may be biased by study design weaknesses. For judging the relevance of minocycline as a potential treatment option in FXS, it will eventually be necessary to weigh the potential benefits against potential side effects, especially in children for whom long-term treatment would be required.

In an open-label, pilot, add-on trial, treatment of 15 patients with FXS (aged 6–23 years) with lithium, which is an inhibitor of regulatory proteins downstream of FMRP, over 8 weeks showed some improvement of behavioral problems, as measured, for example, by the total ABC-C score and cognitive abilities; although the primary end point, the ABC-C irritability subscale, showed only a trend toward improvement [72]. Treatment was relatively safe, with polyuria/polydipsia and elevated TSH as frequent side effects. It remains to be determined whether these observations can be confirmed in larger placebo-controlled trials and whether potential benefits are sufficient to recommend long-term treatment, despite the well-known potential side effects of this drug.

Early experience with targeted treatments in other neurodevelopmental disorders with features of ASD

For the vast majority of ASD cases, which are usually sporadic, the molecular etiology remains unknown despite recent advances in tools and techniques for molecular genetic diagnostic. However, for a subset of patients (~10%) causal mutations in a single gene can be identified, usually accompanied by other neurological and/or neuropsychiatric symptoms. Besides FXS, less frequent examples of such 'syndromic' forms of autism with significant phenotypic overlap to ASD include the TSC and RTT, which are caused by mutations in either the *TSC1* or *TSC2* genes or in the *MeCP2* gene, respectively (reviewed in [2]). As a common theme, proteins encoded by these genes play a key role in regulating the expression of large sets of other proteins, including synaptic proteins that are of particular importance with regard to ASD. Hamartin and tuberin, the proteins encoded by *TSC1* and *TSC2*, respectively, are part of a protein

complex that inhibits intracellular signal transduction to the downstream effectors of mTOR, which regulates the synthesis of many other proteins. The MeCP2 protein, which is responsible for RTT has been shown to be a transcription factor controlling (presumably silencing) the transcriptional activity of various other genes involved in the development of cortical structures. Recent convergent data from various animal models indicate that overactivity of the mTOR-signaling pathway, which is important for both maturation of synapses during brain development and synaptic plasticity in adulthood, may play an important role in the pathogenesis of all three abovementioned 'syndromic' forms of ASD, such as FXS, TSC and RTT [73–76] (reviewed in [2]).

TSC is inherited in an autosomal–dominant fashion and is characterized by benign tumors (hamartomas) or other lesions affecting multiple organs including the skin, brain, kidney, heart, liver and lungs. Cerebral manifestations frequently determine morbidity and mortality (reviewed in [77]). Treatment of mice with a heterozygous inactivating mutation in the *TSC2* gene [*tsc2* (+/-)] with rapamycin, an inhibitor of mTOR, over several days was shown to result in amelioration of several aspects of the TSC phenotype in these mice, that is, cognitive deficits associated with hippocampal-dependent learning, specifically contextual discrimination and spatial learning tasks [78]. These results prompted the initiation of clinical trials with rapamycin (sirolimus) and other mTOR inhibitors (everolimus, temsirolimus and deforolimus) in children with TSC for treatment of various phenotypic manifestations of TSC (e.g., [115]; reviewed in [79]). Recently published results for the treatment of TSC-associated benign tumors are indeed promising: a multicenter double-blind, placebo-controlled study in 117 TCS patients demonstrated that treatment with everolimus resulted in an at least 50% reduction in the volume of subependymal giant cell astrocytomas (the primary end point) in 27 (35%) of the patients versus none in the placebo group, with mouth ulceration and stomatitis as the most frequent side effects [80].

RTT is a severe X-linked neurodevelopmental disorder primarily affecting girls. Typical clinical features include intellectual disability and a mixed movement disorder with dystonia, ataxia, stereotypic hand movements, epileptic seizures and other features, which may include autism [81]. However, it should be noted that the phenotypic spectrum of RTT is highly variable, and that autistic features, even if they are present at any developmental stage (which is usually early), may diminish or even disappear during the course of the disease. In a genetically modified mouse model with a functional *MeCP2*-null mutation in which expression of the MeCP2 protein could be reactivated by exposure to tamoxifen (using the Cre-loxP technique), it was shown that the RTT phenotype in these mice could be reversed by reactivation of MeCP2 expression [82], demonstrating that absence of MeCP2 during development apparently does not irreversibly damage affected neurons, despite the severity of associated symptoms. In another mouse model of RTT with CNS-specific deletion of *MeCP2*, systemic treatment (intraperitoneal injection) of mice with an active peptide of IGF-1, which is known to promote synaptic maturation and neuronal survival, resulted in a partial reversal of the RTT phenotype with extended life span, improved locomotor function, increased brain weight and enhanced synaptic maturation, with increased density of PSD-95 and stabilized cortical plasticity [83]. These results prompted initiation of an ongoing Phase I/II clinical study with human recombinant IGF-1 in children (aged 2–12 years) with RTT [116]. Taken together, the results of these preclinical studies raise hope that targeted treatments may be able to at least partially reverse symptoms of RTT syndrome in humans, even if treatment is only initiated at advanced stages of the disease.

Future perspective

The recently published and ongoing preclinical and clinical studies discussed above have yielded important new insights into the pathogenesis of FXS and other 'syndromic' forms of autism, as well as opening up new perspectives in options for targeted treatments of these frequently severely incapacitating disorders. Further results, for example, of large clinical Phase III studies with mGluR-receptor antagonists, are eagerly awaited and expected to be published soon. It is predicted that at least some of these targeted therapies will be approved by the US FDA and European regulatory agencies as treatment of FXS in the near future. Some important questions that need to be addressed are:

- Can beneficial synergistic treatment effects be observed upon combined treatment with both mGluR-receptor antagonists and drugs targeting the GABAergic system?

Since mGluR5-receptor antagonists and drugs targeting the GABAergic system, such as arbaclofen, act at different levels of the molecular cascade of events involved in FXS, a synergistic effect of both classes of compounds can very well be hypothesized; however, to the author's knowledge, no data from animal models have currently been published that specifically addresses this question, which would be a prerequisite for future corresponding clinical studies, especially since unexpected side effects of such combination therapies might occur.

- What is the optimal time point/age range to initiate pharmacological treatment of FXS? Is newborn screening (NBS) necessary or appropriate in that context?

This important question certainly needs to be investigated further. The results of the aforementioned study by Michalon *et al.* suggest that, at least in a mouse model, considerable therapeutic effects can be observed even if treatment with mGluR5 antagonists is initiated rather late; however, it is currently unclear if these results can be transferred to humans, and if this also applies to other targeted therapies for FXS [46]. These questions certainly need to be addressed in future clinical trials; if the safety of new targeted treatments is established in adults and young adults, further clinical trials involving children and young children can be considered. Considering NBS, future efforts in animal models to demonstrate additional improvements if targeted treatments are initiated at a very early age will be important. In the author's opinion, the currently available data do not justify a recommendation for general NBS for FXS; however, in accordance with current recommendations of the American College of Medical Genetics, *FMR1* screening should be offered to all preconception or prenatal patients, regardless of family history, and it is recommended that pediatricians should order *FMR1* genetic testing for children with developmental delays (if FXS is considered as a differential diagnosis) as soon as these are identified, in order to avoid 'diagnostic odysseys' for families and to increase chances for early intervention, for example, with behavioral treatment [84]. Specific problems associated with NBS for FXS include the possibility that in rare cases individuals with an *FMR1* full mutation may be identified, who are less likely to develop severe FXS due to absent or only partial methylation status of the full mutation allele (which is not detected by the screening assay), and that *FMR1* premutation carriers may be identified who are at risk of developing FXTAS in late adulthood, but not FXS.

- Which clinical outcome parameters and/or biomarkers are best suited to monitor treatment effects in future large clinical trials?

This is a very difficult and complex question that will be a key issue for success of future clinical trials in FXS. There is certainly an urgent need to develop and validate FXS-specific rating scales, both for behavioral and cognitive aspects. In some recent clinical trials, subscales of the ABC have been defined as primary end points, but *post hoc* analysis in several studies showed that newly developed FXS-specific scales, such as the ABC social avoidance scale, may be more useful. For a more detailed discussion of this issue, see [3] and references cited herein, for example [72,85–88].

- Will it be feasible to develop epigenetic modulators for safe and effective clinical use in order to restore transcriptional and translational activity of the *FMR1* gene?

This therapeutic approach is still in the early stages, with most currently existing compounds, such as Azad-C, used successfully only *in vitro* [60], but too toxic for use *in vivo*. Therefore, novel compounds would need to be developed.

- Can other potential treatment targets be identified, among the plethora of other brain mRNAs, for which expression and/or transport is regulated by FMRP?

Given the very large variety of brain mRNAs interacting with FMRP, it is very well possible that further advancements in the understanding of the basic pathophysiology of FXS may result in new candidate compounds for targeted treatments, given that their relevance has been demonstrated in animal models.

- In which cases should preimplantation genetic diagnosis (PGD) for FXS be recommended?

Advances in PCR amplification methods have recently enabled the use of PGD of FXS, which is technically challenging [89,90]. Currently, PGD for FXS is generally recommended only in families with a known *FMR1* mutation. Undoubtedly, due to the complex mode of inheritance and to the multifaceted phenotypic spectrum of *FMR1*-related disorders, offering competent and comprehensive genetic counselling to affected individuals and families remains extremely important. Due to the complex ethical issues involved, there are huge regional differences in the use of PGD both in general and specifically for FXS, also reflecting local differences in legal regulations.

- Are the results of preclinical studies on 'syndromic' forms of ASD at least partially also applicable to the more frequent 'idiopathic' forms of ASD, and will they possibly lead to new treatment options for these patients?

For this issue, results of further ongoing preclinical studies will be important. ASDs undoubtedly comprise an extremely heterogeneous spectrum of disorders with a range of possible symptoms, therefore it is expected that a subset of 'idiopathic' ASDs may be at least partially

caused by molecular pathways overlapping with monogenetic 'syndromic' forms of ASD, which raises hopes that these patients might also benefit from treatments developed for the more homogeneous monogenetic forms.

Nevertheless, apart from pharmacological treatment, behavioral and educational strategies and interventions will remain highly important to the improvement of the quality of life of FXS patients, possibly combined with targeted pharmacologic treatment. It remains to be seen if the insights gained from clinical trials with targeted treatments in patients with single-gene syndromic forms of ASD, for example, FXS, may also translate into improvements in the understanding of the neurobiology of the much more frequent nonsyndromic forms of ASD, for example, by studying treatment–response patterns in larger patient cohorts, possibly combined with the use of potential biomarkers.

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Executive summary

- Preclinical studies in various FMRP-deficient animal models have confirmed the mGluR theory of fragile X syndrome (FXS), which suggests that the deficits associated with FXS, due to loss of function of FMRP, are at least to a large extent caused by upregulation of proteins synthesized in response to mGluR1/5 activation. This synthesis is physiologically inhibited by FMRP. As predicted, treatment with mGluR antagonists in these animal models restores important behavioral, morphological and cognitive aspects of the FXS phenotype.
- Treatment of *FMR1*-knockout mice with a long-acting mGluR5 inhibitor, CTEP, is able to reverse most FXS-associated phenotypes even if treatment is not initiated until young adulthood, suggesting that these symptoms are not irreversibly determined during early embryonic and/or postnatal development.
- Large-scale, double-blind, placebo-controlled, clinical studies with several mGluR-receptor antagonists in humans with FXS are currently ongoing and expected to be published within the next year. Preliminary published results are promising, with improvement of behavioral problems in a subset of patients after short-term treatment without major safety concerns.
- Preliminary results of an exploratory Phase II study suggest that the GABA-(B)-receptor agonist arbaclofen may also improve behavioral problems and social interaction of FXS patients, with larger Phase III trials currently ongoing.
- Other compounds currently in double-blind, placebo-controlled clinical trials include minocyclin as an inhibitor of MMP9, which has shown encouraging results in a small, open-label, add-on pilot trial.
- Targeted treatments are currently also being developed for other neurodevelopmental disorders with features of autism spectrum disorders, specifically tuberous sclerosis complex and Rett syndrome. Converging data from animal models suggest that specific intracellular signaling pathways, for example, the mTOR pathway, are affected in various syndromic forms of autism spectrum disorders. Results of clinical trials in tuberous sclerosis complex suggest that targeted treatment with mTOR inhibitors (e.g., everolimus) may be effective in reducing the volume of subependymal giant cell astrocytomas.

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