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REVIEW

# Fragile X syndrome: a preclinical review on metabotropic glutamate receptor 5 (mGluR5) antagonists and drug development

**Andreea S. Pop · Baltazar Gomez-Mancilla · Giovanni Neri · Rob Willemsen · Fabrizio Gasparini**

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**Abstract**
*Rationale* Fragile X syndrome (FXS) is considered the leading inherited cause of intellectual disability and autism. In FXS, the fragile X mental retardation 1 (*FMR1*) gene is silenced and the fragile X mental retardation protein (FMRP) is not expressed, resulting in the characteristic features of the syndrome. Despite recent advances in understanding the pathophysiology of FXS, there is still no cure for this condition; current treatment is symptomatic. Preclinical research is essential in the development of potential therapeutic agents.
*Objectives* This review provides an overview of the preclinical evidence supporting metabotropic glutamate receptor 5 (mGluR5) antagonists as therapeutic agents for FXS.
*Results* According to the mGluR theory of FXS, the absence of FMRP leads to enhanced glutamatergic signaling via mGluR5, which leads to increased protein synthesis and defects in synaptic plasticity including enhanced long-term depression. As such, efforts to develop agents that target the underlying pathophysiology of FXS have focused on mGluR5 modulation. Animal models, particularly the *Fmr1* knockout mouse model, have become invaluable in exploring therapeutic approaches on an electrophysiological, behavioral, biochemical, and neuroanatomical level. Two direct approaches are currently being investigated for FXS treatment: reactivating the *FMR1* gene and compensating for the lack of FMRP. The latter approach has yielded promising results, with mGluR5 antagonists showing efficacy in clinical trials.
*Conclusions* Targeting mGluR5 is a valid approach for the development of therapeutic agents that target the underlying pathophysiology of FXS. Several compounds are currently in development, with encouraging results.

**Keywords** Fragile X syndrome · mGluR5 · FMR1 · FMRP · DNA methylation · Epigenetic regulation · AFQ056 · Mavoglurant · Dendritic spines · mGluR5 antagonist

A. S. Pop · R. Willemsen (✉)
Department of Clinical Genetics, Erasmus Medical Center, Room Ee 971a, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands
e-mail: r.willemsen@erasmusmc.nl

B. Gomez-Mancilla · F. Gasparini (✉)
Novartis Pharma AG, Novartis Institutes for BioMedical Research Basel, Postfach, 4002 Basel, Switzerland
e-mail: fabrizio.gasparini@novartis.com

G. Neri
Institute of Medical Genetics, Catholic University School of Medicine, Largo F. Vito 1, Rome 00168, Italy

## Introduction

Martin–Bell syndrome, an X-linked intellectual disability, was first described in 1943 by James Purdon Martin and Julia Bell in multiple male members of a family (Martin and Bell 1943). Years later, in 1969, Herbert Lubs discovered the existence of a break on the X chromosome of affected males (Lubs 1969), which was termed “fragile site” by Frederick Hecht in 1970. This led to the name change from Martin–Bell syndrome to fragile X syndrome (FXS). It was only in 1991 that the gene responsible for FXS was identified on the X chromosome at position q27.3, and named fragile X mental retardation 1 gene (*FMR1*) (Verkerk et al. 1991). In FXS, the *FMR1* gene is silenced, and consequently its gene product, fragile X mental retardation protein (FMRP), has reduced expression or is entirely absent. Lack of FMRP expression appears to be at the core of the intellectual disability and other features characteristic of FXS. The prevalence of FXS with the full mutation and intellectual disability is 1:4,000 in males and 1:6,000 in females (Sherman 2012; Turner et al. 1992), and it is considered to be the leading inherited single-gene cause of intellectual disability and autism.

The main clinical phenotype of FXS is intellectual disability. However, individuals typically present with features that are also common to autism spectrum disorder, including deficits in higher cognitive functions, such as delays in speech and language development, impaired theory of mind, and impaired social and emotional processing (Garber et al. 2008). Other characteristic features of FXS include anxiety, attention deficits, hyperactivity, irritability, and autistic-like behaviors including social deficits and hand-flapping, as well as physical characteristics including hypotonia, hypermobility of joints, and macroorchidism (Garber et al. 2008) (Table 1). Dendritic spine abnormalities have been reported in postmortem neuropathological studies in patients with FXS (Hinton et al. 1991; Irwin et al. 2000, 2001; Rudelli et al. 1985). Neuroimaging studies of patients with FXS revealed increased brain size, larger caudate nucleus, increased size of amygdala and hippocampus, cerebellar vermis hypoplasia (Reiss et al. 1995), and ventricular abnormalities (Schapiro et al. 1995), with the right side of the brain being apparently more affected.

The cognitive, physical, and behavioral phenotypes are relatively easy to observe and measure in patients with FXS. Conversely, the neuroanatomical phenotype is much more difficult to observe as it can only be studied in depth in postmortem brain material. Therefore, animal models that mimic the FXS phenotype have become critical in the search for suitable therapies.

**Table 1** Typical characteristic features of FXS

| Features | |
|---|---|
| Physical | Macroorchidism |
| | Long, narrow face with sunken eyes and malar hypoplasia |
| | Highly arched palate |
| | Large and prominent ears |
| | Flat feet |
| | Hypermobility of joints |
| | Hypotonia |
| Social/emotional/behavioral | Hand flapping |
| | Biting |
| | Hyperactivity |
| | Attention deficits |
| | Anxiety |
| | Irritability |
| | Social deficits |
| Intelligence/learning | Intellectual disability |
| | Language deficits |
| | Working and short-term memory problems |
| | Deficits in executive function |
| | Mathematical and visuospatial abilities |
| Sensory | Epileptic seizures |
| | Sleep problems |

## The *FMR1* gene and its product, FMRP

The *FMR1* gene is located on the X chromosome at position q27.3. It has a length of 40 kb and contains 17 exons (Verkerk et al. 1991). Its 5′ untranslated region contains a CGG repeat with a length varying from 6 to 55 repeats in the general population. In some individuals, both males and females, this repeat can become unstable and can reach a length between 55 and 200 CGG repeats, leading to a so-called premutation (Fig. 1). These individuals are known as carriers with a premutation and have a high risk of developing fragile X-associated tremor/ataxia syndrome. Moreover, 20 % of the females carrying a premutation manifest premature ovarian insufficiency (Brouwer et al. 2009). In the case of individuals with FXS, the trinucleotide repeat length expands beyond 200 repeats (full mutation) (Oberle et al. 1991; Yu et al. 1991).

In affected individuals, cytosine residues in the CGG repeat sequence are methylated, with methylation extending to the 52 CpGs of the *FMR1* promoter (Pieretti et al. 1991). Unmutated *FMR1* alleles are also methylated, but in a region further upstream, separated from the *FMR1* promoter by what appears to be a "boundary" that prevents methylation from spreading downstream (Naumann et al. 2009). This boundary is missing in full mutation alleles, and methylation occurs upstream of the CGG repeat region around the 13 weeks of embryonic development (Malter et al. 1997). As a consequence, *FMR1* transcription is inhibited, leading to a reduction in or absence of FMRP from early on during development (Sutcliffe et al. 1992). There are very rare alleles that remain unmethylated despite containing >200 CGG repeats. These alleles maintain some transcriptional activity (Smeets et al. 1995; Tabolacci et al. 2008b; Tassone et al. 2000) and produce reduced levels of FMRP, compatible with "normal" intellectual development. In the case of premutation carriers, FMRP is produced but at a reduced level and, paradoxically, elevated levels of *FMR1* messenger ribonucleic acid (mRNA) are produced (Tassone et al. 2007).

The epigenetic status of full mutation alleles is also characterized by deacetylation of histones H3 and H4, reduced methylation of lysine 4, and increased methylation of lysine 9 (H3K9) on histone H3 (Tabolacci et al. 2008b). These epigenetic changes promote a heterochromatic configuration that excludes the binding of specific transcription factors (Kumari and Usdin 2001), thus turning gene transcription off (Coffee et al. 1999). Notably, the rare unmethylated full mutation alleles maintain a normal epigenetic code, except for H3K9 status, which has methylation levels between normal and full mutation alleles, possibly explaining the reduced transcriptional level of unmethylated full mutations (Smeets et al. 1995; Tabolacci et al. 2008b).

The *FMR1* gene has been highly conserved throughout evolution. Two autosomal paralogs have been identified, fragile X-related genes 1 and 2 (*FXR1* and *FXR2*), located on

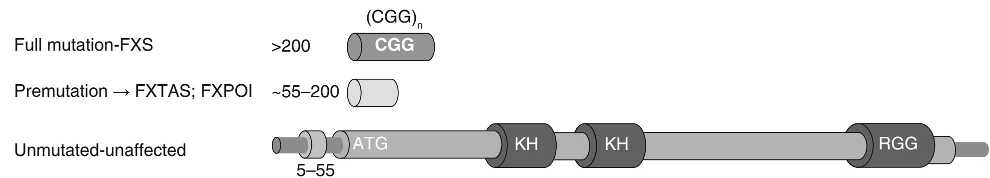


**Fig. 1** The unaffected, permutated, and mutated CCG repeat of the *FMR1* gene

chromosomes 3q28 and 17p13, respectively (Coy et al. 1995; Siomi et al. 1995; Zhang et al. 1995). Together the three genes form the FXR family. There is a high sequence similarity between *FMR1* and *FXR1/2*, especially in their functional domains (Fig. 2) and overlap in tissue distribution. Despite this, FXR1P and FXR2P do not seem to be able to compensate for the lack of FMRP, suggesting that the FXR proteins may have different functions (Coffee et al. 2010).

FMRP is a protein with four major isoforms between 70 and 80 kDa, expressed in many tissues, but predominantly in the brain. High levels are found in the hippocampus and cerebellum, and there is moderate expression in the cerebral cortex (Abitbol et al. 1993; Devys et al. 1993; Hinds et al. 1993; Khandjian et al. 1995). FMRP has also been shown to bind to nitric oxide synthase 1 transcripts during a specific period in human embryonic development (Kwan et al. 2012) that is important for the normal development and function of the nervous system, especially in processes like speech production, language recognition, attention, complex social behaviors, decision making, and emotional processing. In neurons, FMRP is localized mainly in the cell cytoplasm (Devys et al. 1993), where it binds to target mRNAs, including its own mRNA, and travels throughout the cell, and in and out of the nucleus (Devys et al. 1993; Feng et al. 1997; Ferrari et al. 2007; Willemsen et al. 1996). Importantly, FMRP travels into the dendrites via large RNA granules containing target mRNAs, motor proteins, other RNA binding proteins, and ribosomal subunits (de Diego et al. 2002). Target mRNAs of FMRP include: postsynaptic density (PSD)95 (Zalfa et al. 2007), SAPAP1-3 (Brown et al. 2001; Schutt et al. 2009), α-CaMKII, Arc/Ar3.1 (Kao et al. 2010; Zalfa et al. 2003), Shank1 (Schutt et al. 2009), and many more (Darnell and Richter 2012). FMRP regulates the local translation of these mRNAs into proteins at the synapse in the PSD. This process regulates the morphology of the spine and the functionality of the synapse (synaptic plasticity). FMRP also acts as a translational repressor of target mRNAs encoding proteins that regulate the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Fig. 3a), which are essential in the proper function of the synapse.

## The mGluR theory

Aberrant signaling via group 1 metabotropic glutamate receptors (mGluRs), is implicated in the pathophysiology of FXS (Bear et al. 2004). Group 1 mGluRs include mGluR1, expressed mainly in the cerebellum, thalamus, and CA3 hippocampal region and metabotropic glutamate receptor (mGluR)5), highly expressed in the CA1 and CA3 hippocampal regions, cortex and striatum (Dhami and Ferguson 2006; Lujan et al. 1996). FMRP regulates synaptic protein synthesis by binding to ribosomes and stalling translation of target mRNAs (Darnell et al. 2011). In 1997, the first connection between the FMRP and mGluR pathways was identified by Weiler et al. (1997) who observed that activation of group I mGluRs with 3,5-dihydroxyphenylglycine stimulated protein synthesis in synaptoneurosomes including the expression of FMRP (Weiler et al. 1997). In later studies in *Fmr1* knockout (KO) mouse models, Huber et al. showed that the absence of FMRP leads to increased protein synthesis and altered synaptic plasticity, including enhanced long-term depression (LTD) (Huber et al. 2002). These observations led to the formulation of the mGluR theory (Bear et al. 2004), which states that the absence of FMRP in FXS results in excessive glutamatergic signaling via mGluR5. Consequently, this leads to increased local mRNA translation at the synapse, because FMRP is not present to regulate the process, and a high rate of AMPAR internalization and subsequent degradation, which in turn weakens the synapse (Fig. 3b). Increased internalization of AMPARs results in an increased number of longer immature

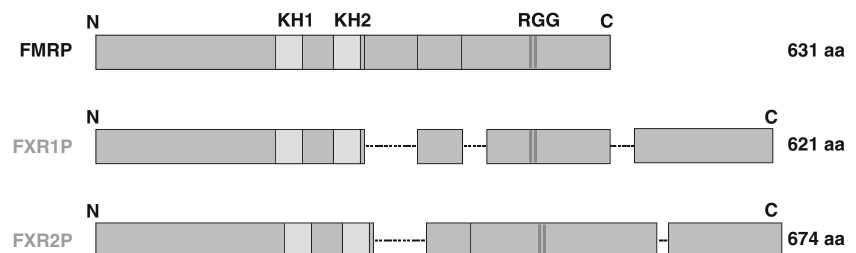


**Fig. 2** FMR1P and its paralogs FXR1P and FXR2P. The KH domains and the C-terminal RGG box are the RNA-binding domains

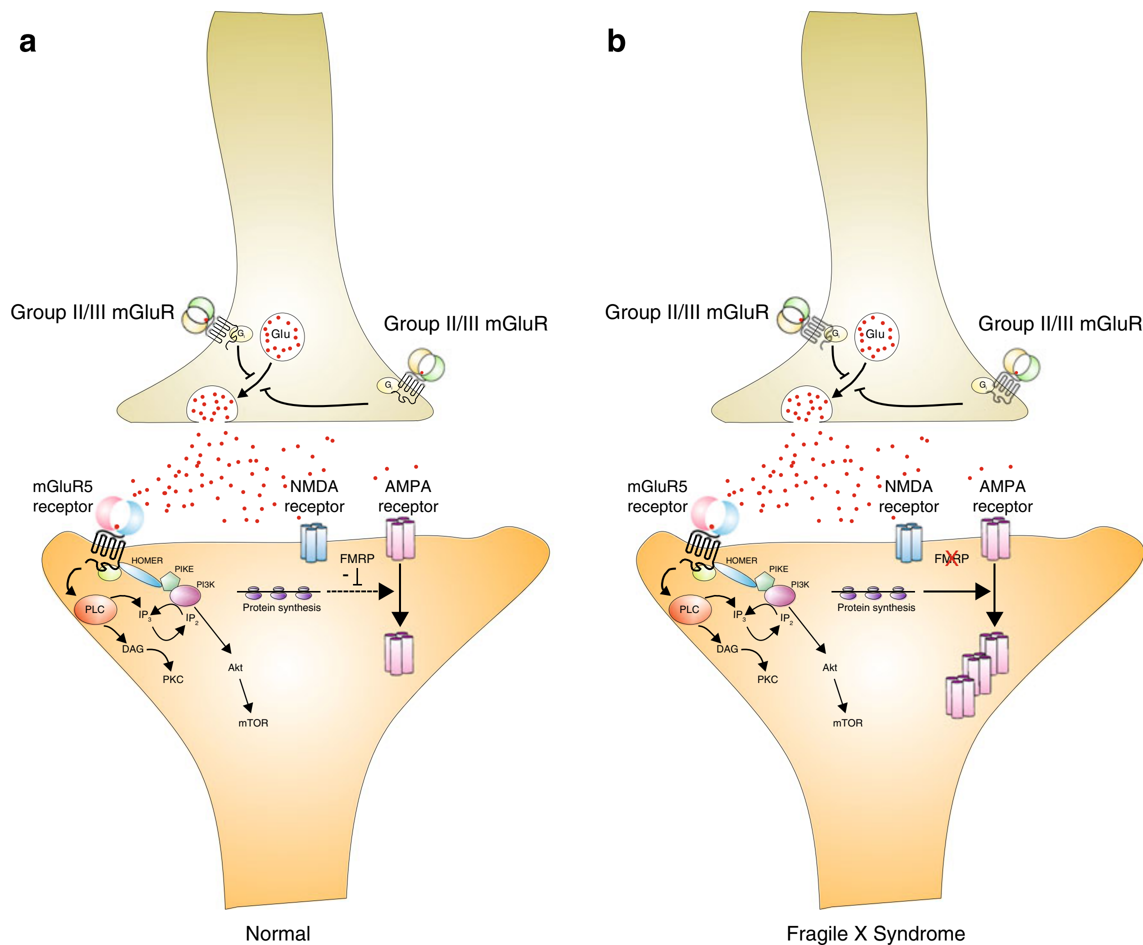


**Fig. 3** Role of FMRP at the synapse—the mGluR theory: **a** unaffected—FMRP present and **b** FXS—absence of FMRP

dendritic spines, which could explain the intellectual disability found in patients with FXS. This immature spine morphology has been observed in both patients with FXS and in animal models mimicking FXS (Portera-Cailliau 2012).

## Transgenic animal models for FXS

To date, preclinical FXS studies have been performed on fruit fly (*Drosophila melanogaster*) (Dhami and Ferguson 2006; Dockendorff et al. 2002; Gatto and Broadie 2008; Kanellopoulos et al. 2012; Pan et al. 2004; Zhang et al. 2001), zebrafish (*Danio rerio*) (den Broeder et al. 2009; Tucker et al. 2004; Tucker et al. 2006; van't Padje et al. 2005), mouse (*Mus musculus*), and lately a rat model (*Ratus norvegicus*; SAGE Labs).

The most widely used animal model for FXS is the laboratory mouse (*M. musculus*). Several mouse models have been generated, such as *Fmr1* KO, *Fmr1* conditional KO, *Fmr1* conditional restoration (Bakker et al. 1994; Mientjes et al. 2006), and recently a mouse model for the I304N mutation, *Fmr1* I304N (Zang et al. 2009). All these lines are available in different strains, such as A/J, C57Bl/6, 129/Ola, FVB, Balb, DBA, and many more (Paradee et al. 1999; Pietropaolo et al. 2011; Spencer et al. 2011). The wide variety of mouse strains (Mouse Genome Database 2012) offers more possibilities for studying different phenotypic aspects of the syndrome, as each strain has different genetic characteristics. However, the interstrain differences sometimes lead to different results for the same aspect studied, and therefore, it is important to choose the correct strain for each study. Additional factors that may contribute to the differences in findings include the region studied, age of the mice and the method used. Despite these differences, it is generally concluded that the *Fmr1* KO mice have abnormal dendritic spine morphology and increased spine density (for a recent review, see Portera-Cailliau 2012), similar to that found in patients with FXS (Hinton et al. 1991; Irwin et al. 2001; Rudelli et al. 1985).

Clinical evaluation scales used to assess cognitive and behavioral impairments in humans cannot be applied to mice. Consequently, several behavioral tests applicable to mice were developed to test processes like learning and memory, such as: T-maze, Morris water maze, fear conditioning test, object discrimination test and many more. *Fmr1* KO mice have been

found to exhibit decreased anxiety in open field; a higher latency to enter a dark box (Michalon et al. 2012); impairment in the acquisition of a visuospatial discrimination task (Krueger et al. 2011) and showed reduced freezing behavior to training context and sound (Guo et al. 2011). In addition, fear conditioning seems to be normal in *Fmr1* KO on a C57BL/6 background, but impaired in KO mice with a C57xFVB background (Paradee et al. 1999), indicating that amygdala function could differ between strains. The same strain difference could account for the variation in results obtained with the Morris water maze, where some studies found impairment in the reversal trials in *Fmr1* KO mice (Bakker et al. 1994; D'Hooge et al. 1997), while others have not observed any difference between KO and wild-type mice in learning and reversal tasks (Paradee et al. 1999). Other behavioral experiments showed that *Fmr1* KO mice exhibited repetitive behavior (measured mainly by marble burying), abnormal social behavior (measured by the three chamber automatic test, or direct/indirect social interaction tests), and audiogenic seizures and anxiety deficiency (in open field or open-arms plus maze, dark–light box); however, results varied between studies (Bilousova et al. 2009; Chen and Toth 2001; de Vrij et al. 2008; Gantois et al. 2013; McNaughton et al. 2008; Michalon et al. 2012; Mineur et al. 2002; Moy et al. 2009; Nielsen et al. 2002; Peier et al. 2000; Pietropaolo et al. 2011; Restivo et al. 2005; Spencer et al. 2005; Spencer et al. 2011).

The abnormal neuroanatomical, cognitive, and behavioral phenotypes found in the *Fmr1* KO mice have been investigated further at the molecular and functional level. Huber et al. showed that a form of LTD, dependent on mGluRs, is altered in the *Fmr1* KO mouse model (Huber et al. 2002). This form of LTD is normally protein synthesis-dependent, but in the case of *Fmr1* KO mice it occurs independently (Nosyreva and Huber 2006; Ronesi and Huber 2008). As mentioned above, the mGluR theory connects FMRP with long-term potentiation and LTD, mainly with increased internalization of AMPARs (Bear et al. 2004). Thus, several studies on the *Fmr1* KO mice have looked at the levels of AMPAR subunits, N-methyl-D-aspartate receptor (NMDAR) subunits and mGluR5 and also at other postsynaptic proteins. Normal levels of AMPAR units GluA1 and GluA2/3 have been found in homogenate preparation from the cortex of 1-week-old *Fmr1* KO mice, but reduced levels in the synaptoneurosome (SNS) fraction; whereas at 2 weeks of age, only the GluA1 subunit was reduced in SNS, while GluA2/3 and GluN2B levels were reduced in homogenates (Till et al. 2012). Giuffrida et al. reported normal levels of AMPA, NMDA and mGluR5 receptors in total protein homogenates and synaptic membrane preparations from the forebrain of *Fmr1* KO mice (Giuffrida et al. 2005). However, homogenates and SNS fractions from the prefrontal cortex of KO mice had decreased levels of NR1, NR2A and NR2B subunits of NMDAR, SAPAP3, PSD-95, and Arc proteins (Krueger et al. 2011). FMRP loss of function has also been linked to GABAergic inhibition in FXS. A decreased level of mRNA for 8/18 $GABA_A$ receptor subunits have been found in the brains of *Fmr1* KO mice (D'Hulst et al. 2006), and reduced levels of $GABA_A$ β subunit levels have been observed in the hippocampus and brainstem compared with control values (El et al. 2005). This may provide an explanation for amygdala dysfunction seen in *Fmr1* KO mice.

FXS animal models, particularly the *Fmr1* KO mouse model, have become invaluable in exploring therapeutic approaches in this field. However, there are some limitations of the KO mouse in modeling the human FXS. In the mouse model the *Fmr1* gene is knocked out from conception, thus, FMRP is not expressed in any cell at any point during development (Oostra and Nelson 2006). Conversely, in humans, the *FMR1* gene is methylated and silenced during embryonic development; therefore, some FMRP is expressed during the very early stages (Willemsen et al. 2002). Moreover, patients frequently present with mosaicism due to the presence of cells (neurons) containing a premutation (size mosaics; ~50 % of patients with FXS) and because the *FMR1* gene is not methylated in all cells (methylation mosaics) (Stöger et al. 2011). Finally, murine lines are inbred and genetically uniform, thus, they are a poor model for FXS in the human population and in particular for FXS treatment studies. Consequently, the genetic differences between humans with FXS and the corresponding murine KO model affect the extrapolation of the preclinical results found through mouse research to patients.

## Considerations for drug development

There are several reasons that justify cautious optimism in finding an effective therapy that targets the underlying pathophysiology of FXS: the condition is a single gene disorder and genetically homogeneous, with very few exceptions; we have detailed knowledge of *FMR1* gene structure; the open reading frame of the mutant gene remains intact, its transcription is stopped by reversible epigenetic changes; we have detailed knowledge of the consequences of the lack of FMRP at the level of dendritic post-synapses; and the clinical condition does not seem to entail irreversible damages to the CNS. For a recent review of potential therapeutic interventions, see Levenga et al. (2010) and Tranfaglia (2011).

The search for FXS targeted therapies was initiated following the identification and characterization of the genetic defect causing FXS (Verkerk et al. 1991). Two direct approaches are currently being investigated for FXS treatment: (1) reactivation of the affected gene and (2) compensating for the lack of FMRP. Restoring *FMR1* gene activity is based on the concept that the epigenetic changes that block transcription are potentially reversible. The idea is to convert a nonfunctional

methylated full mutation to a functional unmethylated full mutation. This approach has made significant contributions to the understanding of the genetic, epigenetic and protein translation mechanism in FXS. Two compounds, 5-Aza-deoxycytidine (Chiurazzi et al. 1999; Tabolacci et al. 2005) and valproic acid (Tabolacci et al. 2008a), have been shown to reactivate the *FMR1* gene to some extent in fragile X cells. In a small, open-label trial of 10 boys with FXS, treatment with valproic acid resulted in a general improvement of hyperactivity and attention deficit, as measured by the Conners scale (Torrioli et al. 2010), although no increase in the mRNA levels of *FMR1* could be measured.

The second approach is based on current knowledge of the signaling pathways impaired by the lack of FMRP, especially within the dendritic post-synaptic vesicles. Pharmacological and genetic rescue studies were mainly inspired by the mGluR theory of FXS. The rationale for the use of mGluR antagonists to treat FXS is strengthened by an elegant study in which mice heterozygous for the *Grm5* gene (encoding mGluR5) were crossed with *Fmr1* KO mice. The resulting 50 % reduction in mGluR5 protein levels led to the correction of some of the typical FXS phenotypic features, especially of the audiogenic seizures (Dölen et al. 2007).

The search for selective mGluR5 antagonists was initiated in 1992 following the cloning of the receptor by the team of S. Nakanishi (Abe et al. 1992). The aim was to identify agents which selectively inhibited mGluR5 and were tolerated in vivo. The first candidates identified were amino acid derivatives that did not distinguish between mGluR1 and mGluR5. The properties of these early agents did not allow them to be considered for further development or for use as pharmacological tools.

Significant progress in the understanding of the physiological role of mGluR5 and the potential therapeutic applications of mGluR5 ligands was made following the identification of the first potent and selective, noncompetitive antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and its precursor molecules SIB1757 and SIB1893 (Gasparini et al. 1999; Varney et al. 1999). Following the discovery of MPEP and publication of its detailed mode of action (Pagano et al. 2000), drug discovery programs were initiated involving industry and academic research laboratories. These led to the identification of a number of candidate mGluR5 antagonists that are currently in preclinical and clinical development (Lindsley and Emmitte 2009; Rocher et al. 2011). Treatments with MPEP in *Fmr1* KO mice resulted in suppression of the audiogenic seizure phenotype (Thomas et al. 2012; Yan et al. 2005), rescuing of the prepulse inhibition (PPI) (de Vrij et al. 2008) and a reduction in repetitive-like behavior (Burket et al. 2011; Thomas et al. 2012). In addition, following MPEP administration (2 weeks), the immature morphological phenotype of pyramidal neurons in the somatosensory cortex of *Fmr1* KO mice was clearly rescued in neonate mice and less effective in 6 weeks old mice (Su et al. 2011). Very recently, Michalon et al. demonstrated in mice that chronic treatment with the novel long-acting mGluR5 antagonist, CTEP, starting at 4 weeks of age could restore cognitive deficits, auditory induced seizures, aberrant dendritic spine density, overactive ERK and mammalian target of rapamycin (mTOR) signaling, and partially corrects macroorchidism (Michalon et al. 2012). Clinically, fenobam, developed previously as an anxiolytic (Pecknold et al. 1982), was the first mGluR5 antagonist tested in FXS. Beneficial effects included reduced anxiety and hyperarousal, improved PPI, and better accuracy on a performance task (Berry-Kravis et al. 2009). Preclinical results of fenobam treatment showed improved motor learning deficiency on the Erasmus Ladder in mice (Vinueza Veloz et al. 2012), and rescuing of the dendritic spine abnormality of *Fmr1* KO cultured neurons in vitro (de Vrij et al. 2008). In a recently completed study, Gantois et al. demonstrated that long-term treatment with AFQ056/mavoglurant, a selective mGluR5 antagonist, can rescue aberrant social behavior in the *Fmr1* KO mice (Gantois et al. 2013). Furthermore, a recent clinical trial of mavoglurant identified a responder subgroup which reported significant improvements in Aberrant Behavior Checklist-Community Edition total score (−27.8 vs. placebo; $P<0.001$), despite no significant improvements in the overall population (Jacquemont et al. 2011). The responder subgroup consisted of patients described as completely methylated according to a bisulfite-sequencing-based method; more sensitive than the widely used southern blot analysis. Patients who are partially methylated showed varying responses to treatment. An active effect on methylation was excluded, as treatment of FXS cell lines with mavoglurant was not found to result in either demethylation or transcriptional reactivation of the *FMR1* gene (Tabolacci et al. 2012). Further clinical trials, currently underway, may provide a better understanding of the mode of action of mavoglurant and severity of the disease, especially with respect to the methylation pattern of patients.

Indirect approaches include targeting signaling pathways downstream or upstream of mGluRs, for example by decreasing the level of glycogen synthase kinase 3β, linked to group I mGluR signaling, which is upregulated in FXS (Min et al. 2009). This theory was supported by the use of lithium in a pilot study trial on 15 patients with FXS. Results showed that 2-month treatment with lithium had positive effects on behavioral adaptive skills (Berry-Kravis et al. 2008).

Currently, the most advanced investigational therapeutic interventions aim to modulate synaptic transmission, either through the reduction of synaptic excitability using selective mGluR5 inhibitors or through the reduction of neurotransmitter release via the activation of the presynaptic $GABA_B$ receptors. Such agents have been developed through large efforts in preclinical research and the use of model organisms

such as the *Fmr1* KO mouse model, as well as established and validated clinical evaluation scales (Sansone et al. 2012).

Additional approaches, currently being investigated preclinically in the *Fmr1* KO mouse model, aim to modulate intracellular targets such as phosphoinositide 3-kinase (PI3K) (Gross et al. 2010), MTOR (Hoeffer et al. 2012), or MAP2K1 and MAP2K2 (MEK 1/2) (Wang et al. 2012). These agents are likely to be investigated in emerging cellular models involving the reprogramming of patient tissue samples in inducible pluripotent cells with a subsequent differentiation in neuronal cells (Sheridan et al. 2011). This novel approach has the potential to improve the validation of biological hypotheses as well as to investigate the effects of new agents without compromising patient safety.

## Conclusions

The monogenic cause of FXS leads to a relatively genetically homogeneous patient population, and offers a unique and favorable situation for research towards developing effective therapies. It also facilitates the use of a variety of transgenic animal models mimicking the FXS phenotype. Although these models do not completely reflect the true human FXS phenotype, they are invaluable for research, understanding the pathophysiology of FXS, and particularly for assessing novel therapeutic approaches.

Despite a genetically homogeneous population, individuals with FXS display significant heterogeneity in clinical phenotype and drug response. A possible explanation might be variance in the epigenetic regulation of the *FMRI* gene and differences in the residual levels of FMRP. However, it is not completely clear how these differences at the molecular level reflect in the overall clinical phenotype.

Research on pharmacological therapies for FXS has been mainly focused on mGluR5 antagonists. Preclinical data from animal research on these agents are encouraging, and there are positive signs from clinical trials in patients with FXS. The results from phase III mavoglurant trials are eagerly awaited and, if positive, could quickly lead to the registration of the first therapy to specifically target the underlying pathophysiology in an intellectual disability syndrome.

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