Fragile X syndrome (FXS) is the most common form of inherited mental retardation and the most common known cause of autism. It is caused by the expansion of a CGG trinucleotide repeat in the 5′ untranslated region of the fragile X mental retardation 1 (FMR1) gene, which encodes the fragile X mental retardation protein (FMRP). FMRP negatively regulates group 1 (Grp 1) metabotropic glutamate receptor (mGlu a.k.a. mGluR) activity, and many FXS phenotypes are thought to be due to the overactivity of the Grp 1 mGlu, mGlu5. This review evaluates the evidence for mGlu5 as a potential therapeutic target in the treatment of FXS. A 50% reduction in mGlu5 expression in Fmr1 knockout (KO) mice has been shown to reverse many FXS-relevant phenotypes including alterations in synaptic plasticity, increased dendritic spine density, increased basal hippocampal protein synthesis, inhibitory avoidance extinction and susceptibility to audiogenic seizures. A negative modulator of mGlu5 may, therefore, be expected to have the same effect. In Fmr1 KO mice, Grp 1 mGlu antagonists, such as 2-methyl-6-(phenylethynyl)pyridine (MPEP), fenobam and AFQ056, have been shown to reduce audiogenic seizures, reverse altered dendritic spine morphology, reduce excessive protein synthesis and improve behavioural abnormalities. MPEP, however, has failed to reverse altered long-term potentiation in the sensory neocortex or reduce macroorchidism. Clinical trials of mGlu5-negative modulators have had some positive outcomes but have had too few participants and were not performed over a long enough period to detect significant effects. Nevertheless, the prospects for development of mGlu5-negative modulators as FXS therapeutics are good and most research supports mGlu5 as a potential therapeutic target.

Key words: fragile X syndrome, mGlu5, mGlu theory, Fmr1, FMRP

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Introduction

FXS is an inherited form of mental retardation first described by Martin and Bell (1943) and was determined to be X-linked dominant with reduced penetrance after a large-scale segregation analysis by Sherman et al. (1984, 1985). Using scanning electron microscopy, Harrison et al. (1983) found the X-chromosomal variant to be located at locus position Xq27.3. Verkerk et al. (1991) became the first to clone the responsible gene: FMR1, which encodes the fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein mainly present in the brain and testes and has been associated with polyribosomes (Ashley et al., 1993b; Khandjian et al., 1996). FMRP is a protein synthesis regulator at synapses, and functions as a translational repressor of target mRNAs (Laggerbauer et al., 2001). It has also been hypothesized that FMRP plays a role in mRNA transportation along dendrites (Bassell and Warren, 2008). In FXS patients, there is a large decrease or complete silencing of the expression of the FMR1 gene, indicating that its loss of function is responsible for the syndrome.

The causative mutation in FXS is a CGG expansion in the 5′ untranslated region of the FMR1 gene (Ashley et al., 1993a). Normal individuals have a CGG trinucleotide repeat of between 6 and 54 (averaging 30 CGG units), while
individuals with 55–200 have a fragile X premutation (Fu et al., 1991). Individuals with >200 CGG repeats have the fragile X phenotype, females (XX) to a lesser extent due to compensation from the other, unaffected X chromosome. These CGG repeats lead to hypermethylation of the surrounding sequence including an upstream CpG island, causing the silencing of FMR1 expression (Pieretti et al., 1991). FXS shows anticipation, which refers to the number of the trinucleotide repeats increasing from one generation to the next, meaning the risk of FXS increases in successive generations (Fu et al., 1991).

FXS is the most common inherited form of mental retardation and is also responsible for 2–6% of autism cases, making it the most common single gene mutation to cause autism (Reddy, 2005; Hagerman, Hoem and Hagerman, 2010). Recent estimates of prevalence using DNA-based diagnostic techniques revealed that FXS is present in ~1 in 4500 males and ~1 in 9000 females, with the premutation present in ~1 in 1000 males and ~1 in 400 females; it is present in every ethnic group (Crawford, Acuña and Sherman, 2001).

In males, FXS is characterized by moderate-to-severe mental retardation and autistic behaviours (Merenstein et al., 1996). There is also a developmental delay, and the majority of males suffer from attention deficit hyperactivity disorder (ADHD) (Hagerman et al., 2009). Another neurological phenotype present in 10–20% of boys with FXS and ~5% of girls is epilepsy, usually first occurring between the ages of 4 and 10 and ending later in childhood (Berry-Kravis, 2002; Incorprora et al., 2002). A connective tissue dysplasia results in the physical symptoms of FXS (narrow face, large ears, macroorchidism and hyperextensible fingers) and it has been hypothesized that hypothalamic dysfunction leads to the development of short bodies and limbs (Loesch et al., 2003). In childhood, ~10% males with FXS develop Prader-Willi phenotype that consists of obesity and hyperphagia (de Vries et al., 1993; McLennan et al., 2011).

There is no cure for FXS, and the core symptom of mental retardation is currently untreatable. However, some other symptoms of FXS may be improved pharmacologically or by behavioural interventions. α-adrenergic receptor agonists are used to treat the ADHD and selective serotonin reuptake inhibitors have been shown to be successful for treating anxiety in over 50% of cases (Hagerman, Murphy and Wittenberger, 1988; Hagerman et al., 1994, 2002). A single anticonvulsant is often used to treat the epilepsy and antipsychotics can help stabilize mood (Berry-Kravis and Potanos, 2004; Hagerman et al., 2009). There are no pharmaceuticals to treat the Prada-Willi phenotype, so the patient’s diet and exercise must be regulated (Balko, 2003; Chen et al., 2007). Due to the lack of cure there is a clear need for new and better therapies for FXS. This review evaluates the evidence for metabotropic glutamate receptor 5 (mGlu5) as a potential therapeutic target in the treatment of FXS, and discusses how this might be achieved.

The mouse model

The mouse Fmr1 gene is 97% homologous to the human FMR1 gene (Ashley et al., 1993b). The first animal model of FXS was created in 1994 by the Dutch Belgian fragile X Consortium through the knock out (KO) of the Fmr1 gene in mice (Bakker et al., 1994). FMRP was shown to be absent in the Fmr1 KO mice by western blotting and the phenotype appears consistent with, yet less severe than, human FXS; the mice had mild behavioural abnormalities, mild mental retardation and macroorchidism. Most mouse models have been created in the same way: by KO of the Fmr1 gene and therefore do not include the expanded CGG trinucleotide repeat and so do not precisely model the causative mutation in FXS. The methylation state of the FMR1 promoter region depends on the number of CGG repeats; Fmr1 KO mice only model the fully methylated form of FXS, which represents only a fraction of humans with FXS (Jacquemont et al., 2011). However, since KO of Fmr1 and the silencing of FMR1 by CGG expansion in human FXS both result in the lack of expression of FMRP the mouse model is sufficiently similar to be useful in FXS research.

Mouse models of FXS do not replicate the anatomical abnormalities found in the brains of FXS patients. The size of the posterior vermis of the cerebellum in humans suffering from FXS is significantly reduced, but there is no difference in the size of the posterior vermis in Fmr1 KO mice when compared with WT littermates (Kooy et al., 1999). However, Fmr1 KO mice and humans with FXS both have more dense dendritic spines which tend to be ‘immature’ (long and thin) in appearance, but there is disagreement in the literature as to the precise phenotype (Irwin, Galvez and Greenough, 2000; Snyder et al., 2001; Vanderklish and Edelman, 2002).

One reason that this mouse model of FXS has been accepted so readily is the broad range of behavioural phenotypes that it reproduces (Bakker et al., 1994). FXS is a complex disease with various neurological symptoms, many of which are expressed in the KO mice, including seizure susceptibility, social behaviour, sensitivity to stimuli and cognitive performance (Musumeci et al., 2000; Dölen et al., 2007; de Vrij et al., 2008). Behavioural phenotype assays have thus far shown small effects in Fmr1 KO mice compared with humans with FXS. This may be because conventionally hippocampus-dependent learning tasks, such as the Morris water maze, have been used, whereas a recent study indicates that prefrontal cortex-dependent learning tasks could reveal larger effects of Fmr1 KO (Krueger et al., 2011).

The mGlu theory of FXS

Mouse models have been instrumental in many breakthroughs in FXS research, revealing FXS as a disorder of long-term depression (LTD) and long-term potentiation (LTP) in the cerebellum, the hippocampus and the prefrontal cortex (Huber et al., 2002; Li et al., 2002; Koekkoek et al., 2008). A connective tissue dysplasia results from FXS is significantly reduced, but there is no difference in the size of the posterior vermis in Fmr1 KO mice when compared with WT littermates (Kooy et al., 1999). However, Fmr1 KO mice and humans with FXS both have more dense dendritic spines which tend to be ‘immature’ (long and thin) in appearance, but there is disagreement in the literature as to the precise phenotype (Irwin, Galvez and Greenough, 2000; Snyder et al., 2001; Vanderklish and Edelman, 2002).

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The activation of both the N-methyl-D-aspartate (NMDA) receptors and the Group (Grp) 1 mGlus results in decreased postsynaptic 2-amino-3-[(methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) and NMDA receptors due to internalization (Oliet, Malenka and Nicoll, 1997). One major mechanical dissimilarity between these two pathways is that the mGlu-dependent LTD involves immediate mRNA translation at the synapse, whereas NMDA-dependent LTD only requires mRNA translation if the LTD continues for more than a few hours (Huber, Kayser and Bear, 2000; Manahan-Vaughan, Kulla and Frey, 2000). mGlu-dependent LTD is also irreversible and entails the loss of the synapse, unlike NMDA-dependent LTD which can be readily reversed (Oliet, Malenka and Nicoll, 1997; Snyder et al., 2001).

The study of FMRP in mGlu-dependent LTD was started when it was discovered that the activation of mGlus promoted the synthesis of FMRP in synaptoneurosomes (Weiler et al., 1997). mGlu-dependent LTD in Fmr1 KO mice was significantly increased compared with wild-type (WT) littersmates but there was no difference in NMDA-dependent LTD, indicating that the phenotype is exclusively linked to mGlu-dependent synaptic plasticity (Huber et al., 2002). mGlu5 is the most prominent Grp 1 mGlu in the forebrain and thus it was hypothesized that mGlu5 activation stimulates the synthesis of proteins which enhance LTD, and FMRP inhibits further synthesis of these proteins, therefore controlling the LTD (Fig. 1A) (Bear, Huber and Warren, 2004). Excessive synthesis of these proteins, as occurs in FXS, leads to internalization of AMPA and NMDA receptors, causing the formation of abnormally long and thin dendritic spines (Fig. 1B) (Irwin et al., 2000; Snyder et al., 2001; Vanderklish and Edelman, 2002).

### Mouse models and the mGlu theory

mGlu-dependent LTD was implicated in FXS by Huber et al. (2002). Paired-pulse facilitation and 3,5-dihydroxyphenylglycine (DHPG) were independently used to induce LTD in separate hippocampal slices of Fmr1 KO mice and WT littersmates. The NMDA antagonist D-APV was used to prevent NMDA-dependent LTD and so the experiment selectively recognized mGlu-dependent LTD. In the case of both the paired-pulse facilitation and DHPG, mGlu-dependent LTD was significantly enhanced in Fmr1 KO mice but not in WT littermates, indicating that mGlu5 is overactive in FXS.

This finding led to the hypothesis that the inhibition of mGlu5 may be a potential therapy for FXS (Bear, Huber and Warren, 2004). This was further investigated by Dölen et al. (2007), who generated Fmr1 KO mice with a 50% reduction in mGlu5 expression. This is possible because the genes for FMRP and mGlu5 (FMRI and GRM5) have single functional homologues in mice (Fmr1 and Gmr5). By crossing Fmr1 KO mice with Gmr5 KO mice, offspring were produced with Fmr1 KO and 50% reduced mGlu5 expression. Complete KO of mGlu5 causes impaired brain function and so it was important to study Gmr5 heterozygous mice with only a 50% reduction in mGlu5 (Fmr1−/−; Gmr5+/−) (Lu et al., 1997). Reducing mGlu5 expression by 50% in Fmr1 KO mice led to the rescue of various FXS-related phenotypes, including synaptic plasticity alterations, increased dendritic spine density, increased basal hippocampal protein synthesis, exaggerated inhibitor avoidance extinction and audiogenic seizure susceptibility. This evidence supports the mGlu theory as well as the notion that pharmaceutical inhibition of mGlu5 could have a similar effect. However, the 50% reduction in mGlu5 expression in Fmr1 KO mice did not reverse the macrophagoidism found in FXS (Dölen et al., 2007), indicating that mGlu5 does not control testicle size. This raises the possibility that there may be other FXS phenotypes that are not rescued by reduced mGlu5 activity. Postnatal pharmacological inhibition of mGlu5 may not have the same effect as genetic inhibition of mGlu5 in FXS patients and mouse models because sufficient damage may have been done already during prenatal development to make the FXS phenotype irreversible.

### Pharmacological inhibition of mGlu5 in mouse models of FXS

One behavioural phenotype that is present in both humans with FXS and Fmr1 KO mice is heightened sensitivity to sensory stimuli. This has been shown to be related to mGlu signalling and can be tested using prepulse inhibition (PPI), a technique in which a reduced startle response is recorded from a stimulus after a weaker prestimulus (Grauer and Marquis, 1999). de Vrij et al. (2008) studied PPI of acoustic startle responses in Fmr1 KO and WT mice. In WT mice, PPI was 73%, whereas it was 30% in Fmr1 KO mice, evidence of the heightened sensitivity in the KO mice. Fmr1 KO mice treated with 20 mg/kg of the mGlu5 inhibitor 2-methyl-6-(phenylethynyl)pyridine (MPEP) 30 mins before the experiment displayed PPI of 70%, an apparent rescue of the phenotype (de Vrij et al., 2008). However, Frankland et al. (2004) recorded increased PPI in Fmr1 KO mice compared with WT mice. This variation could be due to different experimental techniques, since de Vrij et al. (2008) measured the eyelid startle response, whereas Frankland et al. (2004) measured the whole-body startle response. Moreover, while de Vrij et al. (2008) found that MPEP increased PPI in WT mice, this effect on PPI has not been observed in rats treated with MPEP (Henry et al., 2002; Zou et al., 2007).

MPEP has also been shown to reduce audiogenic seizures, which are characteristic of Fmr1 KO mice (Yan et al., 2005). MPEP reduced audiogenic seizures in both Fmr1 KO and WT mice with three different genetic backgrounds (FVB/NJ, C57BL/6j and an F1 hybrid of the two), although the dose was larger in the KO mice. Application of MPEP also rescued the open field phenotype, MPEP-treated Fmr1 KO mice spent as much time as WT mice in the centre of the open field arena. These results support mGlu5 inhibition as a potential therapy in FXS. Importantly, Yan et al. (2005) also discovered that mice can develop a tolerance to MPEP with repeat.
administration of the drug, which could prove to be a problem when developing mGlu5 antagonists as FXS treatments.

Although the Yan et al. (2005) study appeared to show that MPEP reverses two FXS-relevant phenotypes in Fmr1 KO mice, there could be another explanation for its effects. It is conceivable that MPEP is reducing excitatory glutamnergic activity in the auditory pathway, thereby reducing the effect of the stimulus causing the seizures. If this were the case, MPEP may impair auditory acuity but would not be targeting the core symptoms of FXS.

The de Vrij et al. (2008) study also determined the effect of mGlu5 antagonists MPEP and fenobam on dendritic spine morphology in Fmr1 KO mice. The morphology and density of dendritic spines has become a common test for the efficacy of pharmaceuticals in FXS. Although it is recognized that there is some alteration in dendritic spine morphology in FXS.
and Fmr1 KO mice, the literature is not in agreement on the specific alterations. It is generally accepted that the ratio of ‘immature’ long thin spines (filopodia) to ‘mature’ mushroom shaped spines is greater in FXS and in Fmr1 KO mice and that the density of the spines increased (Fig. 2) (Irwin, Galvez and Greenough, 2000). However, studies have produced results varying from reduced spine density in hippocampal cultures of Fmr1 KO mice (Braun and Segal, 2000) to hugely dense filopodia (3–5 filopodia per 10 µm) (Antar et al., 2006). These differing results could arise from varying definitions of ‘immature spines’ and the subjectivity behind their identification. To make the classification of these filopodia more objective, de Vrij et al. (2008) considered all spines with length greater than the width to be filopodia and any width greater than or equal to the length to be mature spines. In this way, de Vrij et al. (2008) found that the density of mature spines was the same in both WT mice and Fmr1 KO mice, but in the KO mice the density of filopodia was significantly greater. After treatment with the mGlu5 agonists MPEP or fenobam, the ratio of normal spines to filopodia matched those of the WT mice. The results of this study conform to the predictions made by Dölen et al. (2007), who rescued the increased dendritic spine density by reducing mGlu5 expression.

The mGlu theory postulates that this altered spine morphology is due to increased protein synthesis, which is regulated by FMRP and stimulated by mGlu5 activation. FMRP binds with up to 4% of the brain’s mRNA in dendrites, including its own, and suppresses its translation to protein (Weiler et al., 1997; Laggerbauer et al., 2001). Translation of FMRP therefore acts as a negative feedback mechanism to stop excess translation caused by mGlu5 activation (Huber et al., 2002) (Fig. 1A). Due to the lack of expression of FMRP in FXS, there is no negative feedback. The function of FMRP in mRNA translational regulation is incredibly complex and diverse, with much controversy surrounding its precise role. FMRP has been shown to be a translational activator as well as a repressor; it plays a part in translation initiation and elongation as well as being associated with the RNA-induced silencing complex. Many of the mRNAs that FMRP binds to, such as MAP1B, are factors in spine morphology (Gross, Berry-Kravis and Bassell, 2012).

Aschrafi et al. (2005) observed decreased mRNA granules (shown to contain FMRP by western blot of rat brain lysate) in the brains of Fmr1 KO mice compared with WT. When treated with 35 mg/kg of MPEP, the size of the mRNA granules were significantly increased in both Fmr1 KO and WT mice. This suggests that granules are stabilized by FMRP and destabilized by mGlu5 activity. This study does not directly measure protein synthesis; the link between mRNA granules and level of protein synthesis is only hypothetical. It is unclear why Aschrafi et al. (2005) performed a western blot of brain lysate from rats to prove the presence of FMRP in mRNA granules: the study does not show that FMRP is present in mRNA granules in mice brains.

This increased protein synthesis leads to altered synaptic plasticity. In Fmr1 KO mice, the most characterized form of altered synaptic plasticity is enhanced mGlu5-dependent LTD in the Shaffer collaterals of the CA1 region of the hippocampus (Huber, Roder and Bear, 2001; Huber et al., 2002). To date, no studies have investigated the effects of mGlu5-negative modulation on LTD anywhere in the brain. However, the effect of the Grp 2 mGlu antagonist LY341495 on mGlu5-dependent LTD in Fmr1 KO mice has been researched in CA1 neurons (Choi et al., 2011), the rationale being that antagonism of DmGluA, the Drosophila flies sole mGlu homologue, has been shown to rescue memory and social interaction (McBride et al., 2005). DmGluA has both Grp 1 and 2 mGlu activity. Chronic treatment with the Grp 2 mGlu antagonist LY341495 for 8 weeks rescued DHPG-induced LTD in adult Fmr1 KO mice, but increased DHPG-induced LTD in WT mice. The reason for the contrasting effects in Fmr1 KO and

![Figure 2. Spine morphologies of Golgi-impregnated neurons.](https://academic.oup.com/biohorizons/article-abstract/doi/10.1093/biohorizons/hzt001/301323)
WT mice is unknown but Choi et al. (2011) hypothesized that it could be due to some sort of compensation in the system. However, LY341495 is not completely selective for Grp 2 mGlu, as it is also a Grp 3 and a Grp 1 mGlu antagonist. LY341495 has a 1000-fold greater affinity for Grp 2 mGlu than Grp 1, and 10-fold greater for Grp 2 than for Grp 3 (Kingston et al., 1998). The dose was also kept at the lowest level previously shown to reverse the in vivo effects of a Grp 2 mGlu agonist (Johnson et al., 1999). Nevertheless, it cannot be ruled out that the antagonistic effect on the Grp 1 or Grp 3 mGlus did not cause the phenotype rescue.

In contrast to LDP, LTP is reduced in the sensory neocortex of Fmr1 KO mice but not in the hippocampus (Fig. 3) (Li et al., 2002). Wilson and Cox (2007) showed that in vitro LTP in the visual neocortex in WT mice is not only NMDA-dependent but primarily due to mGlu activity. They proposed that the reduced LTP measured in the visual cortex of Fmr1 KO mice is due to reduced mGlu5 activity. When the mGlu5 inhibitor MPEP was applied to the visual cortex of Fmr1 KO mice, there was no significant difference in LTP between MPEP-treated and control Fmr1 KO mice. This suggests that mGlu5-mediated synaptic plasticity is in deficit in the visual cortex, in contrast to the hippocampus where mGlu5-mediated synaptic plasticity is in excess. If this is the case, it poses serious problems for mGlu5 as a potential therapeutic target; since it shows that the relationship between FMRP and mGlu5 is more complicated than originally thought. It also implies that FMRP-regulated events may be specific to particular brain areas, rendering mGlu5 inhibition useless or perhaps even harmful in some areas of the brain.

Yan et al. (2005) postulated that mGlu5-negative modulators, such as MPEP, will only have an effect on phenotypes caused by FMRP-independent signalling and may have no effect on FMRP-dependent signalling, thus, some FXS phenotypes will remain (Fig. 4). Essentially, the only FMRP function that mGlu5-negative modulators would rescue may be the regulation of mGlu5 activity.

MPEP is not a specific inhibitor of mGlu5, and can inhibit NMDA receptors when at high concentrations (Lea, Movsesyan and Faden, 2005), thus raising the possibility that MPEP treatment could potentially have adverse effects. Most studies have tried to use MPEP at concentrations low enough not to affect NMDA activity, but it is conceivable that some conclusions drawn from research involving MPEP could be due to its NMDA-inhibiting action rather than its ability to inhibit mGlu5. MPEP cannot be used as a therapy in humans with FXS owing to its toxicity. It also has a very short half-life (~1 h in C57BL/6J mice, Anderson et al., 2003) meaning that the administration of the drug would have to be more frequent than is practicable.

AFQ056 is a drug developed by Novartis which could potentially be used to treat FXS in humans since it does not have the same drawbacks as MPEP, such as toxicity and short half-life. Levenga et al. (2011) tested the effect of AFQ056 on the spine morphology and PPI of Fmr1 KO mice. AFQ056 was shown to restore PPI in Fmr1 KO mice from 22 to 48% and also increased PPI in WT mice. AFQ056 also affected spine morphology: when administered to Fmr1 KO mice in three different concentrations (10 nm, 1 µM and 10 µM), the hippocampal dendritic spine length decreased. Interestingly, AFQ056 increased spine density and decreased spine width. This is in contrast to the results of the in vivo study by Dölen et al. (2007), in which a decrease in the spine density and an increase in the spine width was observed when mGlu5 expression was reduced by 50% in Fmr1 KO mice. As a relatively new drug, very little research has been conducted using AFQ056. There have not yet been any studies published that confirm AFQ056 as a selective mGlu5 antagonist as it is described by Levenga et al. (2011). Much more research is needed before it can be considered a potential therapy.

Figure 3. Graph showing the LTP response to tetanic stimulation in WT and in Fmr1 KO mice. Taken from deep layers of the visual neocortex in the presence of low concentration of the GABA	extsubscript{A} antagonist bicuculline. LTP is significantly reduced in the Fmr1 KO mice compared with the WT mice over 60 min of tetanic stimulation. MPEP did not rescue the reduced LTP in Fmr1 KO mice (data not shown). Taken from Wilson and Cox (2007); Copyright 2007 National Academy of Sciences, USA.
In recent years, the positive results of studies into mGlu5-negative modulators in preclinical animal models has led to the development of pharmaceuticals and clinical trials in humans with FXS that target mGlu5, including fenobam, AFQ056 (Novartis), STX107 (Seaside Therapeutics) and RG7090 (Hoffmann-LaRoche).

A pilot open label, single-dose trial of fenobam was conducted on 12 subjects (6 male, 6 female) with FXS (Berry-Kravis et al., 2009). The main aim of the experiment was to determine the safety of the drug and identify any significant adverse effects, of which there was none. Each patient received a single dose of fenobam (between 50 and 150 mg) and was then screened for vital signs and side effects during the following 6 h. 50% of the patients had amended PPI after drug administration ranging from 23.7 to 44.2% improvement. It is known that the placebo effect is exaggerated in individuals with mental retardation (Sandler, 2005) and so the open label nature of this trial may have some influence on the results. The results also suffered due to the limited number of patients in the trial and the fact that they were only given a single dose. However, the lack of adverse effects is promising for future trials.

A double-blind, two-treatment, two-period crossover trial of the subtype-selective mGlu5 inhibitor AFQ056 was conducted on 30 individuals with FXS (Jacquemont et al., 2011). Individuals were screened to determine the extent of the methylation of their FMR1 promoter. Interestingly, only individuals with a fully methylated promoter and no FMR1 mRNA detected in the blood showed significant improvements in comparison with the control group when measured using various behavioural rating scales. This suggests that screening for full methylation could determine which patients would benefit from mGlu5 antagonists.

The response to treatment in patients with partial methylation of the FMR1 promoter was varied. Jacquemont et al. (2011) propose that this variation in response is due to differences in methylation states and therefore to different degrees of mGlu5 hyperactivity (Fig. 5). The baseline scores of the behavioural rating scales indicate a more severe phenotype in the fully methylated participants which supports this theory; however, this difference is not statistically significant. The lack of correlation between FMR1 mRNA in the blood and response to AFQ056 casts doubt over this hypothesis, although it could be explained by variance in tissue-specific methylation or variance in the translation of FMR1 mRNA to FMRP. An assessment of FMRP levels may have helped draw conclusions as to why there was so much variation in response amongst the partially methylated subpopulation but no such analysis was carried out. The small number of trial patients (seven with fully methylated FMR1 promoters) makes it difficult to draw any solid conclusions from this study. Future trials with more participants may offer clues to the mechanism behind why fully methylated participants responded more consistently to treatment.

Jacquemont et al. (2011) chose to use behavioural response to measure efficacy of AFQ056. Measurement of change in PPI and other behavioural measures following treatment with AFQ056 was compared with baseline levels to determine efficacy. A schematic showing the influence of the FMR1 promoter methylation state on PPI improvement is provided in Figure 5.
in PPI or eye tracking would have been a more objective way to quantify the response. Also, to see a developmental improvement in the participants rather than just symptomatic improvements, future trials will have to be conducted over significantly longer time periods, particularly in older patients.

Currently, there are other mGlu5-negative modulators such as STX107 and RG7090, which are undergoing clinical trials but the result have yet to be released.

**Discussion**

Much of the evidence in preclinical animal models of FXS indicates that mGlu5-negative modulators may eventually be used therapeutically. The studies using mouse models are summarized in Table 1. There are minor inconsistencies within these studies, many of which could be due to differing experimental techniques. Taken collectively, however, these works tend to support the mGlu theory put forward by Bear, Huber and Warren (2004) but do not paint a complete picture. Some results indicate that Grp 2 mGlus may also be implicated in the mGlu theory (Wilson and Cox, 2007; Choi et al., 2011). The major limitation within the preclinical studies is the fundamental problem of modelling human diseases in animals. The validity of the models depends on the extent to which the animal disease is analogous to the human disease. In the mouse models, the behavioural phenotype is less obvious and the symptoms much milder than in human FXS. Overall, the prospect for the development of mGlu5-negative modulators as a therapy appears good, as is evident from the clinical trials now underway.

One important question in need of answering is whether or not the effect of FXS during prenatal development is reversible by pharmaceuticals. The application of mGlu5-negative modulators is unlikely to be a practical option as the average age of diagnosis is at ~3-5-37 months in males and later in females (Bailey et al., 2009). In most models, it has been shown that at least some of the phenotypes are reversed by treatment with mGlu5-negative modulators in adulthood. The clinical trials during prenatal development that have been performed so far tell us very little about the long-term effects of mGlu5-negative modulators. Future trials need to be carried out over a much longer time period and with many more participants to find out to what extent these drugs can be beneficial.

Twenty-four of the 30 participants in the Jacquemont et al. (2011) study experienced adverse effects, mostly fatigue and headaches but some suffered from hyperlipasemia, hyperamylasemia, increased hepatic enzymes and increased blood creatinine phosphokinase. At higher doses these adverse effects may become more severe and as this was a relatively small trial there may be other more dangerous side effects during prenatal development that have been performed so far tell us very little about the long-term effects of mGlu5-negative modulators. Future trials need to be carried out over a much longer time period and with many more participants to find out to what extent these drugs can be beneficial.

Table 1. Summary of studies of mGlu5-negative modulation in Fmr1 KO mice

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Reversed phenotype</th>
<th>Target</th>
<th>Study</th>
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<tr>
<td>Genetic rescue; 50% reduction in mGlu5 expression</td>
<td>Altered synaptic plasticity, dendritic spine density, basal hippocampal protein synthesis, inhibitor avoidance extinction, audiogenic seizures</td>
<td>mGlu5</td>
<td>Dölen et al. (2007)</td>
<td>50% reduction in the mGlu5 expression was unable to rescue macroorchidism</td>
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<tr>
<td>MPEP</td>
<td>Decreased PPI of acoustic startle response</td>
<td>Grp 1 mGlus</td>
<td>de Vrij et al. (2008)</td>
<td>Inconsistent with the findings of Frankland et al. (2004) who observed increased PPI in Fmr1 KO mice compared with WT. Hugely varying alterations in spine morphology was observed in Fmr1 KO mice (Braun and Segal, 2000; Antar et al., 2006)</td>
</tr>
<tr>
<td>MPEP, fenobam</td>
<td>Increased ratio of filopodia to mature dendritic spines</td>
<td>Grp 1 mGlus</td>
<td>de Vrij et al. (2008)</td>
<td>As above.</td>
</tr>
<tr>
<td>MPEP</td>
<td>Audiogenic seizures, centre field behaviour</td>
<td>Grp 1 mGlus</td>
<td>Yan et al. (2005)</td>
<td>Reduction in auditory seizures could be due to reduced glutaminergic activity in the auditory pathway decreasing the effect of the stimulus. MPEP was also unable to rescue increased size of testis</td>
</tr>
<tr>
<td>MPEP</td>
<td>Excessive protein synthesis</td>
<td>Grp 1 mGlus</td>
<td>Aschraft et al. (2004)</td>
<td>No evidence that FMRP is present in mRNA granules in mouse brains. Link between the mRNA granule size and the level of protein synthesis is only hypothetical</td>
</tr>
<tr>
<td>LY341495</td>
<td>Increased DHPG-induced LTD in the CA1 region of the hippocampus</td>
<td>Grp 2 mGlus</td>
<td>Choi et al. (2011)</td>
<td>LY341495 has a very small antagonist effect on Grps 1 and 3 mGlus</td>
</tr>
<tr>
<td>MPEP</td>
<td>None</td>
<td>Grp 1 mGlus in sensory neocortex</td>
<td>Wilson and Cox (2007)</td>
<td>MPEP did not rescue excess LTP in the sensory neocortex</td>
</tr>
<tr>
<td>AFQ056</td>
<td>Decreased PPI, increased spine length</td>
<td>Grp 1 mGlus</td>
<td>Levenega et al. (2011)</td>
<td>AFQ056 increased spine density and decreased spine width which is in contrast to Dölen et al. (2007) in which the decreased mGlu5 expression did the opposite</td>
</tr>
</tbody>
</table>
effects that did not present themselves. The results of the Wilson and Cox (2007) study indicate a variance in the mGlu5 activity across different brain regions; this is a potential problem with regard to the therapeutic use of mGlu5-negative modulators. Specifically mGlu5 inhibition has been shown to have no effect on the increased LTP in the visual cortex and may have a negative effect by decreasing visual cortex LTD (Wilson and Cox, 2007).

Inhibition of mGlu5 does not offer a complete cure for FXS, since some symptoms such as machroorchidism have proved to be resistant to mGlu5-negative modulation and there may be other refractory symptoms yet to be identified (Dölen et al., 2007). Perhaps inhibition of mGlu5 will help to control many of the symptoms and, along with other drugs, significantly improve the quality of life of the patient and the carers. With further research into mGlu5-negative modulators in animal models and through clinical trials, their full therapeutic potential will be revealed.

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Author biography

I graduated from the University of Leeds in July 2012 with a degree in neuroscience. My particular interests are neurological disorders and the development of therapies for them. I am currently applying for postgraduate study.

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