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Fragile X syndrome: from molecular genetics to therapy

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ABSTRACT

Fragile X syndrome, the main cause of inherited mental retardation, is caused by transcriptional silencing of the fragile X mental retardation gene, *FMR1*. Absence of the associated protein FMRP leads to the dysregulation of many genes creating a phenotype of ADHD, anxiety, epilepsy and autism. The core aim of this review is to summarize two decades of molecular research leading to the characterisation of cellular and molecular pathways involved in the pathology of this disease and as a consequence to the identification of two new promising targets for rational therapy of fragile X syndrome, namely the group 1 metabotropic glutamate receptors (Gp1 mGluRs) and the gamma-aminobutyric acid A receptors (GABA_ARs). As no current clinical treatments are directed specifically at the underlying neuronal defect due to absence of FMRP, this might open new powerful therapeutic strategies.

THE FRAGILE X PHENOTYPE AND MOLECULAR ASPECTS

Mental retardation, defined as a failure to develop a sufficient cognitive and adaptive level, is one of the most common human lifelong disorders. According to estimates, 1-3% of the human population has an IQ below 70 [1, 2]. Fragile X syndrome is the main cause of inherited mental retardation and the leading known genetic form of autism affecting, according to the latest estimates, 1/2500 individuals [3, 4, 5]. Cognitive dysfunction in fragile X syndrome, includes deficiencies in working and short-term memory, executive function, and mathematic and visuospatial abilities [6, 7, 8]. Next to cognitive impairment fragile X patients show various physical abnormalities such as large testicles (macro-orchidism), connective tissue dysplasia, a characteristic appearance of a long, narrow face, large ears and a close interocular distance, flat feet, and sometimes hyperextensible joints, hand calluses and strabismus.

In addition, speech and language skills are severely affected in males with fragile X syndrome, who often exhibit autistic-like behaviour including poor eye contact, perseverative speech and behaviour, tactile defensiveness, shyness, social anxiety, and hand flapping and biting [9], as well as seizures and EEG findings consistent with

epilepsy [10]. Anxiety and mood disorders, hyperactivity, impulsivity, and aggressive behaviour can also be present [11].

At the cytogenetic level, chromosome spreads of fragile X cells grown under specific cell culture conditions show a gap or break on the X-chromosome. This is the so-called fragile site FRAXA at Xq27.3 [12]. At the molecular level, the fragile site is caused by a CGG triplet expansion (dynamic mutation) to more than 200 repeats located within the 5' untranslated region of the *Fragile X Mental Retardation 1 (FMR1)* gene. The concomitant hypermethylation of the CpG island in the promoter region of the gene causes transcriptional silencing of *FMR1* [13]. In somatic tissue, all cytosine residues in the upstream CpG island become completely methylated. Hypermethylation of the CpG island is followed by histone deacetylation, perhaps in effort to stop the expansion of the repeat [14, 15, 16]. Thus, amplification of the CGG repeat results in a change of the chromatin structure to a very condensed, transcriptionally inactive structure [17]. Smeets et al. [18] reported unmethylated expanded CGG repeats and cytogenically visible fragile sites in two clinically normal brothers, indicating that inactivation of the *FMR1* gene and not repeat expansion itself results in the fragile X phenotype. Thus, repeat expansion does not necessarily induce methylation and methylation is no absolute requirement for induction of fragile sites. Less-affected males typically have partial methylation, resulting in an incomplete activation of *FMR1* and they may have an IQ in the borderline or even in the normal range [19]. Due to X-inactivation, affected females show in general a milder phenotype and the severity of dysfunction is correlated with the degree of lyonisation on the abnormal chromosome.

Normal individuals carry 6 to 54 CGG repeats, while alleles with 55 to 200 triplets are considered 'premutated' genes [20]. The premutation is unstable and commonly expands during intergenerational transmission. Interestingly, the repeat is more stable during male transmission, and the full mutation can only be inherited from the mother [21].

Premutation carriers can develop a late-onset neurodegenerative syndrome called fragile X tremor/ataxia syndrome (FXTAS).

THE FRAGILE X MENTAL RETARDATION GENE, *FMR1*

The *FMR1* gene belongs to a small gene family that includes the *fragile X related gene 1* and 2 (*FXR1* and *FXR2*). *FXR1* and 2 are autosomal genes mapping at 3q28 and 17p13.1, respectively [13, 22]. *FMR1*, *FXR1* and *FXR2* are highly conserved in evolution and orthologues are present in all vertebrates. *Drosophila* has one single related gene, *dFmr1* [23]. Human *FMR1* consists of 17 exons and spans 38 kilobases. The transcript length is 4.4 kb. Two major consensus sites, USF1/USF2 and alpha-Pal/Nrf-1, within the *FMR1* promoter site have been shown to be involved in the positive regulation of *FMR1* expression [24]. Beilina et al. [25] demonstrated that transcription of the *FMR1* gene is initiated at three different start sites (I-III) for both neuronal and non-neuronal cells. They have also observed that the relative utilization of the three principal start sites is significantly altered depending on the size of the expansion of the CGG repeat, indicating that the downstream CGG element has a direct influence on transcriptional initiation. Thus, redistribution of the 5' ends of the *FMR1* message could play a role in the reduced translation efficiency observed for premutation alleles. The *FMR1* promoter is CG-rich and lacks a typical TATA element, but it does contain three initiator-like (Inr) sequences that correspond to sites I-III. Inr sequences are usually located near transcription start sites and have been implicated in transcription initiation from TATA-less promoters [26, 27].

THE FRAGILE X MENTAL RETARDATION PROTEIN

FMRP, structure and expression

FMRP, the protein encoded by the *FMR1* gene, is an RNA-binding protein that is maximally 631 amino acids long. Intensive alternative splicing occurs especially in the 3' terminal half of the gene, in exons 12, 14, 15 and 17. This can potentially give rise to 12 different protein isoforms. FMRP contains two hnRNP K-protein homology (KH) domains and an Arg-Gly-Gly RGG box, which are motifs characteristic of RNA-binding proteins

[28]. Additionally a nuclear localisation signal (NLS) and a nuclear export signal (NES) and two coiled coils (CC) involved in protein–protein interaction have been identified. The G-quartet structure present in the mRNA is believed to interact with the RGG box in the protein (Figure 1).

FMRP is highly conserved among vertebrates and is widely, but not ubiquitously expressed. Particularly high expression is observed in ovary, thymus, eye, spleen and esophageal epithelium with an abundant expression in brain and testis. A moderate expression of FMRP has been demonstrated in colon, uterus, thyroid and liver, but no expression has been observed in the heart, aorta or muscle. In brain, FMRP expression is restricted to differentiated neurons particularly in the hippocampus and granular layer of the cerebellum and is absent in non-neuronal cells [29, 30]. Neuronal FMRP is concentrated in the perikaryon and proximal dendrites. Expression was also detected in synapses but not in axons [31].

Fmrp, RNA targets and protein interactors

The search for RNAs that bind to FMRP (FMRP RNA targets) resulted in the identification of a large number of mRNAs that direct the synthesis of different proteins with a variety of functions. FMRP binds a significant percentage of brain mRNAs and has a preference for two classes of mRNAs that contain either a G-quartet structure or an U-rich sequence [32, 33, 34, 35, 36]. Using a new technique, Miyashiro et al. [37] identified some 80 mRNAs, of which 60% were directly associated with FMRP. In the brain of *Fmr1* KO mice, some of these mRNAs, as well as their corresponding proteins, display subtle changes both in location and in abundance, pointing to a critical role for FMRP in targeting neurospecific mRNAs to the synapse.

FMRP can interact with a range of proteins either directly or indirectly. Using yeast-to-hybrid or co-immunoprecipitation techniques, direct interactions of FMRP with FXR1P, FXR2P, NUFIP1 (nuclear FMRP interacting protein 1), 82-FIP (82 kDa FMRP interacting protein) and microspherule protein 58 (MSP58) have been described [reviewed

by 38]. These proteins might modulate the affinity of FMRP for different classes of mRNAs by inducing structural changes in conformation, thus exposing the RNA-binding domains differentially. Additional RNA-binding proteins such as nucleolin, YB-1/p50, Pur- α and Staufen have been detected in complex structures containing FMRP, but it is not known whether these bind directly to FMRP [39]. Only a few non-RNA-binding proteins have been shown to interact with FMRP, including: the actin-based motor protein myosin Va ; Ran-BPM and Lgl, which are cytoskeleton associated proteins; and CYFIP1 and CYFIP2, which link FMRP to the RhoGTPase pathway .

FMRP and regulation of translation

FMRP is thought to play a key role in synaptic plasticity through regulation of mRNA transport and translational inhibition of local protein synthesis at the synapses [40]. Jin and Warren [41] have proposed a model of FMRP neuronal functioning, which is based on several pathological and biochemical studies. According to that model, dimerized FMRP is transported into the nucleus via its NLS. In the nucleus it assembles into a messenger ribonucleoprotein (mRNP) complex thereby interacting with specific RNA transcripts and other proteins. The FMRP-mRNP complex is transported out of the nucleus via its NES. Once in the cytoplasm, the FMRP-mRNP complex interacts with members of the RNA-induced silencing complex (RISC) before associating with ribosomes. The FMRP-mRNP complex regulates protein synthesis in the cell body of the neuron or the complex could be transported into the dendrites to regulate local protein synthesis of specific mRNAs in response to synaptic stimulation signals such as metabotropic glutamate receptor (mGluR) activation. The accumulation of data suggests that the RNA interference (RNAi) pathway is the major molecular mechanism by which FMRP regulates translation. Specific interactions were observed between dFmrp and two functional RISC proteins, dAGO (Argonaute 2) and Dicer, which mediate RNAi [42]. This raises the possibility that might regulate the translation of its target genes through micro RNAs (miRNA). Endogenous miRNAs are a class of non-coding RNAs, between 18 and 25 nucleotides in length, that are believed to control translation of specific target RNAs by imperfect base-pairing with

complementary sequences in the mRNA 3' untranslated region [40, 43]. Unfortunately, the exact mechanism of the action of FMRP together with RISC is not clear at present. A likely scenario is that once FMRP binds to its specific mRNA ligands, it recruits RISC along with miRNAs and facilitates the recognition between miRNAs and their mRNA ligands. Recent data suggest that one single miRNA can regulate multiple mRNA targets, while a given mRNA can be regulated by multiple miRNAs. This provides transient and temporal translational regulation which allows the translation to be rapid and reversible, a requirement for protein dependent synaptic plasticity.

An additional mechanism by which regulation of translation could occur is through phosphorylation of FMRP, which might modulate the translational state of FMRP [44]. Both mammalian *Fmrp* and *Drosophila dFmrp* can be phosphorylated *in vivo* at a phosphorylation site that is conserved throughout evolution (Ser500 in mammals and Ser406 in *Drosophila*). Thus removal of the phosphate by activated phosphatase might be the signal for *Fmrp* to release the translational suppression and allow synthesis of a locally required protein [45]. Alternatively, FMRP has been proposed to behave as a nucleic acid chaperone [38]. Nucleic acid chaperones bind in a cooperative manner to one or several nucleic acid molecules to favour the most stable conformation, while at the same time preventing folding traps that might preclude function of the target nucleic acid. Once the most stable nucleic acid structure is reached, the continuous binding of the chaperone is no longer required to maintain the structure [46]. Based on its chaperone activities, the binding of one or a few FMRP molecules opens the mRNA structure, favouring the initiation stage for protein translation. Thus, FMRP might regulate translation by acting on the structural status of mRNA, and mRNA transition from a translatable to an untranslatable form would be due to an increase of bound FMRP molecules, including a densely packed structure of the mRNP complex [38].

Fmrp and regulation of mRNA stability

Zalfa et al. [47] reported a new cytoplasmic regulatory function for FMRP: control of mRNA stability. In mice, they found that Fmrp binds the mRNA encoding PSD-95, a key molecule that regulates neuronal synaptic signalling and learning. This interaction occurs through the 3' untranslated region of the *PSD-95* mRNA, increasing message stability. Moreover, stabilisation is further increased by mGluR activation. They suggest that dysregulation of mRNA stability may contribute to the cognitive impairments in individuals with FXS.

FMRP and spine dysgenesis

In neurons, FMRP is localized within and at the base of dendritic spines in association with poly ribosomes. This association is RNA as well as microtubule dependent, indicating a role for FMRP in mRNA trafficking and dendritic development. Dendritic spines are the postsynaptic compartments of mostly excitatory synapses in mammalian brains. There is growing evidence that induction of synaptic plasticity correlates with changes in the number and/or shape of dendritic spines [48]. Dendritic spines in fragile X syndrome are denser apically, elongated, thin, and tortuous [49]. In *Drosophila* too, dFmrp acts as a regulator of cytoskeleton stability, and loss of dFmrp function in neurons results in inappropriate sprouting, branching and growth, causing gross changes in both axon and dendrite projections in motor, sensory and central neurons [50]. Thus loss of FMRP results in altered microtubule dynamics that affect neural development and, therefore, indicates a potential role for FMRP in synaptic plasticity [51, 52]. A link between abnormal dendritic spines and mental retardation has been suggested previously for other cognitive disorders such as Down and Rett syndrome [53].

ANIMAL MODELS

Mouse models (*Mus musculus*)

Fragile X mouse

FMR1 is highly conserved between human and mouse, with a nucleotide and amino acid identity of 95% and 97%, respectively [13, 54]. The expression pattern of mouse *Fmr1* is similar to its human counterpart in both tissue specificity and timing which makes the mouse a good animal model to study FXS [55, 56]. To investigate the function of *FMR1* in mental retardation a mouse was developed in which exon 5 of the *Fmr1* gene is interrupted with a neomycine cassette [57]. Although this insertional mutation is not identical to CGG repeat expansion, it does cause loss of intact *Fmr1* RNA and Fmrp production, like in patients.

The KO mice show deficits in spatial learning, altered sensorimotor integration and mildly increased locomotoractivity [as reviewed by 58, 59]. Physical abnormalities include macro-orchidism which is manifested from day 15 after birth onwards. The increase in testicular weight exceeds 30% at 6 months. One common brain feature of fragile X patients and of the mouse model is the increased spine density and the excess of long and thin immature spines indicative of defective pruning during development [49, 60, 61]. Electrophysiological findings suggest a significant increase in mGluR-dependent LTD in the hippocampus of the knock-out mouse. Because LTP and LTD are commonly believed to be involved in learning and memory, the observed abnormalities might relate to the cognitive deficits observed in FXS [62]. One of the clearest neurological parallels between the mouse model and fragile X patients is an increased susceptibility to seizures [63, 64]. Remarkably, increased seizure susceptibility of fragile X mice is specific to auditory stimuli, as seizure sensitivity of fragile X mice to chemical convulsants (bicuculline, PTZ and kainic acid), when compared to wild types, was not increased [63].

To be able to create *Fmr1* null alleles in specific cell types and at selected points in development, Mientjes et al. [65] generated a *Fmr1* conditional KO by flanking the murine *Fmr1* promoter and its first exon with loxP sites. Similar to *Fmr1* KO1 mice, *Fmr1* KO2, with the first exon constitutively excised, also display macroorchidism with testis weights 18% higher than the WT controls. Typically, the KO2 line generates no *Fmr1* mRNA, whereas in the KO1 line aberrant *fmr1* mRNA, but no *Fmrp* has been observed [57, 66].

CGG repeat model

To better understand the timing and mechanism involved in the *FMR1* CGG repeat instability and methylation, several attempts to make transgenic mouse models with expanded CGG tracts were undertaken [67, 68, 69, 70]. However, since flanking of the expanded CGG repeat with part of the *FMR1* gene proved not sufficient to recapitulate all aspects of repeat instability in humans, the endogenous mouse CGG repeat was replaced by a human CGG repeat carrying 98 CGG units [71]. The inheritance of the CGG repeat is only moderately unstable, upon both maternal and paternal transmission, indicating differences between the behaviour of the *Fmr1* premutation CGG expanded-repeat in mouse and in human transmissions. Mice with repeats up to 230 repeats have been reported [72]. However, although this length is in the full mutation range, methylation is absent, suggesting that modelling the fragile X full mutation requires additional repeats or other genetic manipulation. As in humans, the expanded CGG repeat model shows 2-3.5 fold elevated mRNA levels in brains tissue compared with control. The model displays biochemical, phenotypic and neuropathological characteristics of FXTAS [73]. Importantly, immunohistochemical studies provide significant evidence for the presence of ubiquitin-positive intranuclear inclusions in neurons of this mouse model. Numbers and size of the inclusions increase with age, which parallels with the progressive nature of the disorder in humans. The striking contrast to humans is the absence in the mouse of astrocytic intranuclear inclusions and other neuropathologic features, including neuronal loss, gliosis and marked strop out of Purkinje cells.

Rescue mouse

To determine whether fragile X syndrome is a potentially treatable disorder, several attempts have been made to rescue the silenced murine *Fmr1* [reviewed by 74]. Most successfully, a YAC containing 450 kb of the human Xq27.3 region and the full length of the *FMR1* gene was used to generate a transgenic mouse [75]. Breeding these YAC transgenic lines with *Fmr1* KO mice results in 4 different genotypes: wild type, wild type with the YAC, *Fmr1* KO mice and KO mice harbouring the YAC. Testicular weights were restored within the normal range for the *Fmr1* KO mice carrying the YAC transgene, indicating a functional rescue by the human protein. Partial rescue was observed in behavioural tests and it was evident that the cell specificity as well as the quantity of the FMRP should be strictly regulated. Recently, Musumeci et al. [76] reported that the reintroduction of FMRP is able to partially rescue the audiogenic seizure susceptibility of *Fmr1* KO mice.

Fly model (*Drosophila melanogaster*)

The neurological phenotypes of the *Fmr1* KO mouse are subtle, at both behavioural and cellular levels, which has made it difficult to assess the role of FMRP *in vivo*. In response to this limitation, a *Drosophila* fragile X syndrome model was developed by mutating the homologous *Drosophila melanogaster mental retardation gene 1* (*dFmr1* or *dFxr*) [23, 52]. *DFmrp*, displays considerable amino acid sequence similarity with the vertebrate FMRP, especially within the functional domains. It possesses conserved tissue and subcellular expression patterns, similar RNA-binding capacity, a conserved functional role as translational repressor and is required for normal neurite elongation, guidance and branching [77, 78, 79, 80]. These findings suggest that the *Drosophila* model can complement and expand studies in mice.

Dfmr1 deficient fly models are viable, anatomically normal and display a wide repertoire of apparently normal behaviours. However, *dFmr1* null mutants show significant locomotory defects [52]. More complex behaviours manifest stronger deficits, including

abnormal eclosion and circadian rhythm and aborted courtship ritual. Anomalies in the morphology of several central nervous system neuronal populations have also been observed. Thus, *dFmr1* mutants appear to display more prominent phenotypes than mouse *Fmr1* KO's. This might be due to the presence of *Fxr1* and *Fxr2* in the knockout mouse, whereas *dFmr1* deficient flies have no remaining paralogs of the gene [50].

Zebrafish model (*Danio rerio*)

The zebrafish has a full complement of genes orthologous to the human gene family, as well as *Fmr1* interacting proteins that are crucial to understanding the context-dependent activities of the transcript and protein. Tucker and colleagues [81] established the zebrafish embryo as a model for loss-of-function analysis. Morpholino antisense oligonucleotide repression of *Fmr1* mRNA translation in zebrafish embryos was used to produce changes in neurons and neurite branching in the central and peripheral nervous systems. They demonstrated that the zebrafish is an entirely appropriate and easily manipulated fragile X model in which to examine multiple aspects of the syndrome.

THERAPEUTICAL APPROACHES

Treatment strategies for individuals with fragile X syndrome are at this point rather supportive designed to maximize functioning, as no treatments in current clinical use are directed specifically at the underlying neuronal defect resulting from the absence of FMRP. As behaviour in fragile X syndrome can significantly impact functionality, symptom-based treatment of the most problematic behaviours of the individual can be quite helpful [82]. Based on functional studies, two theories have been put forward upon which experimental therapeutic approaches have been initiated.

The mGluR theory

Synaptic activity in the brain can trigger long lasting changes in synaptic strength called long-term potentiation (LTP) and long-term depression (LTD). These mechanisms work in concert to contribute to learning and memory storage throughout postnatal life. One type of LTD is triggered by activation of postsynaptic group 1 metabotropic glutamate

receptors (Gp1 mGluRs, comprised of mGluR1 and mGluR5), requires rapid translation of pre-existing mRNA in the post synaptic dendrites and stimulates the loss of surface expressed synaptic AMPA and NMDA receptors [83]. Huber et al. [62] reported that mGluR dependent LTD was significantly altered in the hippocampus of *Fmr1* KO mice. Rather than a deficit, however, they found that mGluR-LTD was augmented in the absence of FMRP. This finding is consistent with the discovery that FMRP normally functions as a negative regulator of translation. Based on the evidence that FMRP is normally synthesized following stimulation of Gp1 mGluRs [84], they proposed a simple model to account for this findings [85]. According to this model, mGluR activation normally stimulates synthesis of proteins involved in stabilisation of LTD and, in addition, FMRP. The FMRP functions to inhibit further synthesis and puts a brake on LTD. They hypothesize that exaggerated LTD and/or mGluR function are responsible for several aspects of the fragile X phenotype. Their studies in the fragile X KO mouse revealed that exaggerated LTD could slow net synaptic maturation by tipping the balance away from synapse gain to synapse loss in the critical period of synaptogenesis, and therefore contribute to the developmental delay and cognitive impairment associated with the disease. Bear et al. [85] also relate the net loss of AMPA and NMDA (i.e. elevated LTD) with the elongation of dendritic spines, as seen in fragile X patients. According to this view, elongated spines are weakened synapses en route to elimination, and/or filopodial extensions of dendrites seeking to replace lost synapses. This theory predicts that Gp1 mGluR antagonists have great promise as a potential treatment of the neurologic and psychiatric symptoms of fragile X expressed in adults.

Inspired by this theory, pharmacological and genetic rescue studies have been initiated (Table 1). In flies, Mc Bride et al. [86] demonstrated that treatment with 2-methyl-6-(phenylethynyl)-pyridine (MPEP), an mGluR antagonist, or lithium can rescue courtship and mushroom body defects and restores the memory defects associated with deficits in experience-dependent modification of courtship behaviour observed in *dFmr1*^{-/-} mutant flies. Using a zebrafish model for fragile X syndrome, Tucker et al. [81] showed that

MPEP rescues *Fmr1* loss-of-function neurite branching abnormalities in zebrafish embryos. Additionally it was demonstrated that over expression of *Fmr1* in normal embryos and MPEP treatment have similar effects on neurite branching.

It was reported that acute administration of MPEP, can reversibly suppress seizure phenotypes in fragile X knockout mice [87]. However, the interpretation of this result is complicated by the fact that the drug has an anticonvulsant effect in wild-type mice as well. This group also showed that the administration of MPEP restores the aberrant open field exploratory behaviour they found in the *Fmr1* knockout mice [66]. Another recent study in fragile X mice reported a clear defect in prepulse inhibition of startle that could be restored by MPEP and the rescue of fragile X related protrusion morphology, of dendritic spines cultured *in vitro*, using two different mGluR antagonists, MPEP and fenobam [88]. Fenobam is a selective and potent mGluR5 antagonist, with inverse agonist properties, acting at an allosteric modulatory site shared with the prototypical mGluR5 antagonist MPEP [89]. In contrast to MPEP, robust anxiolytic activity and efficacy of fenobam in humans was already reported in a double blind placebo controlled trial [90].

Using a genetic strategy, Dolen et al. [91] showed unambiguously that FMRP and mGluR5 act as an opponent pair in several functional contexts, supporting the theory that many central nervous systems in fragile X are accounted for by unbalanced activation of Gp1 mGluRs. By crossing *Fmr1* mutant mice with mutant *Grm5* (murine functional homologue of the human gene encoding mGluR5, i.e. *GRM5*) mice, *Fmr1* knockout animals with a selective reduction in mGluR5 expression were generated. A 50% reduction of the mGluR5 receptor in the *Fmr1* knock out mouse rescued many behavioural and structural abnormalities of the *Fmr1* knockout mouse but not the macroorchidism (Table 1).

We can conclude that mGluR5 antagonists offer one target for pharmaceutical intervention in fragile X syndrome. Although no such antagonists are currently available

as approved drugs for use in men, there is reason to be optimistic. Currently, two mGluR5 antagonists are planned to go in clinical trials. STX107 is the lead compound from a series of highly potent and selective mGluR5 antagonists. STX107 is a small molecule invented, patented, and extensively characterized in preclinical assays and behavioral models by Merck scientists. Seaside therapeutics in-licensed this product from Merck and has planned phase I clinical trials starting in 2008 (<http://www.seasidetherapeutics.com/programs/lead-drug.htm>). Dr. Randi Hagerman (UC Davis, CA, USA) reported recently during the FRAXA Investigators Meeting (Durham, NH, USA, Sept 2008) on the first clinical trial of fenobam in fragile X patients (funded by Neuropharm and FRAXA foundation). Though this trial was primarily designed to assess safety of a single dose of the drug, improvements in mood were noted. Also, a physiologic test called pre-pulse inhibition showed improvement in half the patients after only one dose of fenobam (<http://www.fraxa.org/newsrelease4.aspx>).

The GABA_A receptor theory

Several independent lines of evidence suggested involvement of the GABA_A receptor and the GABAergic system in fragile X syndrome [reviewed by 92]. As GABA_A receptors are involved in anxiety, hyperactivity, epilepsy, autism spectrum disorder, insomnia and learning and memory, processes also disturbed in fragile X syndrome, we argued that a dysfunction of the GABAergic system has neurophysiologic and functional consequences that might relate to the behavioural and neurological phenotype associated with fragile X syndrome. Therefore, the GABA_A receptor might be a novel target for treatment of this disorder.

The first pharmacological experimental proof for this theory was reported recently by Chang et al. [93]. They discovered that *Fmr1*^{-/-} mutant *Drosophila* die during development when reared on food containing increased levels of glutamate. Using this lethal phenotype, they screened a chemical library of 2000 compounds and identified 9 molecules that rescued lethality. Interestingly, 3 of them were implicated in the GABAergic pathway.

The pharmacology of the GABA_A receptor is well documented and many GABA_A receptor agonists are readily available or currently in clinical trials. The best known GABAergic drugs are the benzodiazepines (BZD), which enhance GABAergic function. Clinically used BZD agonists, such as diazepam are proven anxiolytics, but they often exhibit undesirable side-effects, including sedation and ataxia and cessation of treatment can cause rebound of anxiety and insomnia [94]. Partial GABA_A receptor agonists retaining the anxiolytic efficacy of existing BZD but devoid of the sedation liability are currently under investigation [95, 96, 97]. A totally different type of drugs are the neuroactive steroids that act as allosteric modulators of the GABA_A receptor. For instance, ganaxolone has a favourable safety profile and is now in phase II clinical trials for promising treatment of catamenial epilepsy [98]. Use of this drug is now also planned for treatment of audiogenically induced seizures in fragile X mice by us. Clinical trials to evaluate the effect of this drug in patients are scheduled [99].

Thus, fragile x syndrome is an example of a disease in which the identification of the causative gene in 1991 led to the characterisation of the cellular and molecular pathways involved in the pathology of the disease, eventually leading to the discovery of two independent targets for rational therapy (Figure 2). It is a hopeful fact that newer targeted psychopharmacological agents such as fenobam and STX107, mGluR5 antagonists, and ganaxolone, a GABA_A receptor agonist, will be used for the first time in clinical trials in fragile X patients in hope to improve the clinical symptoms in patients with fragile X syndrome [99].

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Table1

<i>Experimental proof</i>				
Target	Animal model	Rescued phenotype	Reference	Theory
<i>Pharmacological rescue</i>				
mGluR5	Fly	Courtship behaviour Mushroom body defects Memory defects	McBride et al. 2005 [86]	Huber et al. 2002 [62] Bear et al. 2004 [85]
	Zebrafish	Axonal branching	Tucker et al. 2006 [81]	
	Mouse	Epileptic seizures Open field behaviour Prepulse inhibition of startle Protrusion morphology	Yan et al. 2005 [87] De Vrij et al. 2005 [88]	
GABA _A R	Fly	Lethality (when reared on food containing increased levels of glutamate)	Chang et al. 2008 [93]	D'Hulst and Kooy, 2007 [92]
<i>Genetic rescue</i>				
mGluR5	Mouse	Altered ocular dominance plasticity Increased dendritic spine density Increased basal protein synthesis Exaggerated inhibitory avoidance extinction Audiogenic seizures Accelerated body growth	Dolen et al. 2007 [91]	
GABA _A R	NA			
<i>Planned clinical trials in patients</i>				
Target	Drug			
mGluR5	Fenobam		Cornish et al. 2008 [99] Neuropharm	
GABA _A R	STX107 ganaxolone		Seaside therapeutics Cornish et al. 2008 [99]	

mGluR: Metabotropic glutamate receptor 5; GABA_A R: gamma-amino butyric acid A receptor

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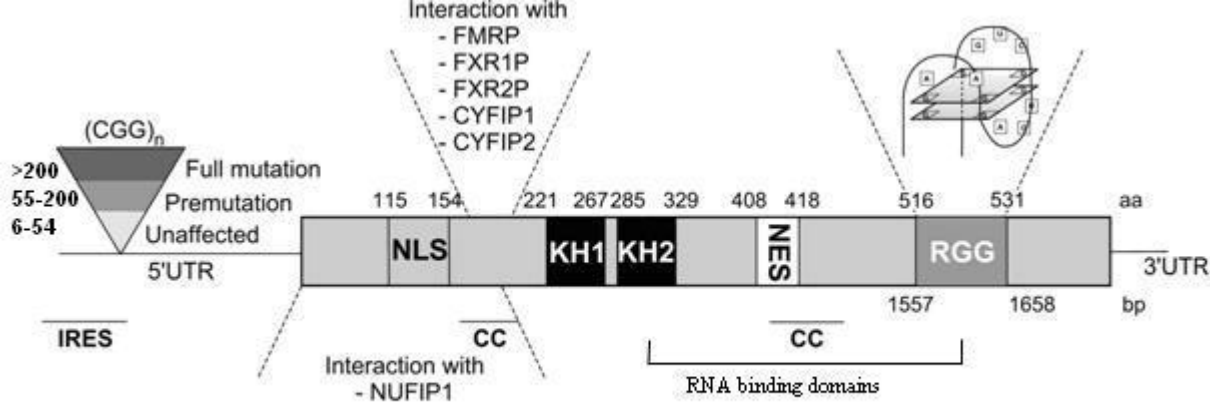
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Legends to figures:

Figure 1: Schematic representation of the *FMR1* mRNA and protein. The known domains are indicated. IRES: internal ribosomal entry site. 5'UTR: 5' untranslated region; NLS: nuclear localisation signal; KH: hnRNP K-protein homology domains; NES: nuclear export signal; RGG: arginine-glycine-glycine; 3'UTR: 3' untranslated region

Figure 2: Fragile X syndrome: from molecular genetics to therapy



Fragile X syndrome: from molecular genetics to therapy

