Fragile X and Autism: Intertwined at the Molecular Level Leading to Targeted Treatments

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Abstract

Fragile X syndrome (FXS) is caused by an expanded CGG repeat (>200 repeats) in the 5' un-translated portion of the fragile mental retardation 1 gene (*FMR1*) leading to deficiency or absence of the *FMR1* protein (FMRP). FMRP is an RNA carrier protein that controls the translation of a number of other genes that regulate synaptic development and plasticity. Autism occurs in approximately 30% of FXS cases, and Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS) occurs in an additional 30% of cases. Premutation repeat expansions (55 to 200 CGG repeats) may also give rise to autism spectrum disorders (ASD), including both autism and PDD-NOS, through a different molecular mechanism that involves a direct toxic effect of the expanded-CGG-repeat *FMR1* mRNA. RNA toxicity can also lead to aging effects including tremor, ataxia and cognitive decline termed the fragile X-associated tremor ataxia syndrome (FXTAS) in premutation carriers in late life. In studies of mice bearing premutation expansions, there is evidence of early post-natal neuronal cell toxicity, manifest as reduced cell longevity, decreased dendritic arborization and altered synaptic morphology. There is also evidence of mitochondrial dysfunction in premutation carriers. Many of the problems with cellular dysregulation in both premutation and full mutation neurons also parallel the cellular abnormalities that have been documented in autism without fragile X mutations. Research regarding dysregulation of neurotransmitter systems in FXS, including metabotropic glutamate receptor 1/5 (mGluR1/5) pathway and $GABA_A$ pathways, have led to new targeted treatments for FXS. Preliminary evidence suggests that these new targeted treatments will also be beneficial in non-fragile X forms of autism.

Introduction

Fragile X Syndrome (FXS) is an important subtype of autism, both because of its frequency and because our knowledge of the molecular mechanisms involved in FXS pathogenesis has facilitated the development of targeted treatments with the potential to reverse or dramatically improve both behavioral and cognitive deficits. Since FXS is the most common single gene cause of autism, responsible for 2 to 6% of all cases of autism, it is clinically recommended that all individuals diagnosed with autism or ASD should have the fragile X DNA test (both PCR and Southern Blot) when the etiology of their autism is not known [1-4]. FXS is nearly always caused by a trinucleotide (CGG) repeat expansion, located in the 5' untranslated region of the *FMR1* gene, to greater than 200 repeats (full mutation range). Full mutation expansions typically lead to methylation of the gene, reduced or absent transcription, and decreased consequent reduction in translation of the *FMR1* protein (FMRP), the proximal basis of FXS. The level of FMRP is correlated with the degree of clinical involvement including physical, cognitive and structural/functional brain involvement [5-10].

Approximately 30% of males with FXS have full autism, as determined by the standardized criteria of the Autism Diagnostic Observation Scale (ADOS) and the Autism Diagnostic Interview (ADI-R) [11-15]. An additional 30% of boys have PDD-NOS [11]. Among the remaining patients with FXS, those who do not meet the criteria for an autism spectrum disorders (ASD) diagnosis, the majority have one or more autistic features, such as hand flapping, poor eye contact, and tactile defensiveness [11].

The premutation CGG-repeat range (55-200 repeats) was initially defined in terms of an increased frequency of expansion of the CGG repeat to the full mutation range when transmitted by a premutation (carrier) female. All children with the full mutation have a carrier mother, although a female with the premutation could have received this mutation from either her mother or her father. Moreover, the propensity for transmission of a full mutation allele increases with increasing CGG repeat number in the mother [16]. A father who is a carrier of either a premutation or full mutation allele will pass only the premutation to all of his daughters, presumably due to selective production of premutation allele-bearing sperm [17].

Carriers of premutation alleles were generally considered to be clinically uninvolved until premature ovarian failure, recently renamed fragile X-associated primary ovarian insufficiency (FXPOI), was reported [18]. Subsequently, the late onset neurodegenerative disorder, fragile X-associated tremor ataxia syndrome (FXTAS), was described [19, 20], further establishing clinical involvement among premutation carriers. It is now evident that a spectrum of neurodevelopmental and aging/neurological problems are associated with the premutation, including autism and ASD [21-26]. Most individuals with the premutation are neither developmentally disabled nor do they have autism; however, a subgroup does experience, cognitive, emotional and/or behavioral involvement.. There is a negative correlation between CGG repeat number and the level of FMRP in the premutation range [27], which predisposes individuals in the high end of the premutation range to cognitive and behavioral impairment. In addition, all individuals with the premutation have elevated *FMR1* mRNA, while the opposite occurs in the full mutation [27]. Thus, the cognitive and behavioral impairments in the premutation and full mutation ranges are likely to have both distinct and overlapping mechanisms.

Clinical and Molecular Involvement in FXS and Association with Autism

The basis for incomplete penetrance of autism (30%) or PDD-NOS (30%) among individuals with FXS is not known. However, there is evidence that patients with additional medical disorders that affect the CNS, such as seizures or additional genetic problems have an increased risk for autism compared to patients with FXS alone [28-30]. For those with both FXS and autism, there is a spectrum of involvement with significant heterogeneity both cognitively and behaviorally, with IQ values ranging from severely intellectually impaired to normal, particularly in females. However, there is a strong association between low IQ and the autism diagnosis in both males and females with FXS [11-14, 31-35]. The cause of this heterogeneity is related to background genetic effects and environmental effects that influence IQ, social abilities, anxiety, ADHD and additional features that are components of the phenotype of FXS (Figure 1). Background genetic effects include additional pathological mutations (FXS has been reported with sex chromosomal disorders, Down syndrome, Tourette syndrome and other conditions [28, 29, 36], allelic variants [37], or gene expression changes [38]). An example of the later condition is the Prader-Willi phenotype (PWP) of FXS where there is no structural or methylation

change at 15q 11-13; rather, there is significant down regulation of expression of CYFIP 1, which is located at the 15q locus of Prader-Willi Syndrome (PWS) [38]. Males with the PWP of FXS have severe obesity, hyperphagia, hypogenitalia, and a higher rate of ASD than those with FXS without the PWP [38].

Environmental influences to the phenotype of FXS include exposures to toxins (e.g. alcohol leading to fetal alcohol syndrome and FXS), abuse (physical or sexual), neglect, perinatal asphyxia, head trauma, seizures and socioeconomic status. Additional environmental exposures leading to further toxicity are just beginning to be explored in both premutation and full mutation involvement, as they are in idiopathic autism [39-42]. Such studies are occurring at a cellular level in the premutation neurons; these neurons die earlier than control neurons, with increased cell death documented by 21 days in culture [43]. In addition, mitochondrial dysfunction has been documented in fibroblasts and brain tissue in premutation carriers both with and without FXTAS [44]. We hypothesize that premutation neurons are more vulnerable to environmental toxins, and clinical case reports appear to support this notion [42, 45].

The absence of FMRP in individuals with FXS has significant consequences in the translation of dozens and likely hundreds of proteins. Since FMRP usually suppresses translation, its absence leads to broad translational up-regulation in the hippocampus [46]. Recent studies by Darnell et al [47] and others have demonstrated linkage between FMRP and many proteins that are related to autism, including Neuroligin 3 and 4, Neurorexin, PSD 95, CYFIP 1 and 2, SHANK 3, Arc, PTEN, MAPKinase, JAKMIP1, HERC2 among others [2, 47-50]. Most of these proteins are associated with synapse formation and plasticity, however, the PTEN (phosphatase and tensin homologue) gene encodes a dual specificity phosphatase effecting G1 cell cycle arrest and /or apoptosis, and 17% (3/18) of individuals with autism and macrocephaly were found to have a PTEN mutation [51] . Macrocephaly also occurs In FXS, often with a broad forehead remarkably similar to the broad foreheads described by Butler and colleagues; this characteristic is hypothesized to be related to down-regulation of PTEN, which occurs in FXS [47, 52]. Expression of Janus kinase and microtubule interacting protein 1 (JAKMIP1) and the G protein coupled receptor 155 (GRP155) were both altered by the reduction of FMRP (seen in FXS) or the induction of CYFIP1 (seen in 15q duplication form of autism) *in vitro*

[53]. These proteins were also dysregulated in boys with idiopathic ASD relative to their unaffected siblings [53]. Both CYFIP1 (a partner protein to FMRP and regulated by FMRP) and JAKMIP1 are involved with the RacGTPase system that modulates the neurite development that is critical for proper brain connectivity [54]. . There is also evidence for up-regulation of the mTOR pathway in the hippocampus of the KO mouse [55] and in studies of humans with FXS [56]. The mTOR pathway is dysregulated in a number of other genetic disorders that are associated with autism, such as tuberous sclerosis (TS) [57]. These findings have stimulated targeted treatments utilizing rapamycin to down-regulate the mTOR system in patients with TS, with initially positive results . The overlap of molecular mechanisms in those with the premutation or the full mutation and idiopathic autism is visualized in **Figure 2**.

Recently a number of studies have compared directly those with FXS and those with autism without FXS. There are unique CNS structural differences between the two disorders even when both disorders have comparable degrees of autism as assessed by standardized behavioral measures [58]. Those with FXS have an enlarged caudate compared to typically developing individuals and those with autism; whereas those with autism have a larger amygdala compared to FXS or controls [58]. These differences continue to evolve with age, as does the severity of the autistic features in FXS [59]. Therefore, from early in life - and likely *in utero*, there are CNS structural changes that are related to the lack of FMRP. The dysregulation of proteins that are important for synaptic plasticity and connectivity in the brain leads to the gradual deficits in socialization, behavior, and cognition that characterize the FXS phenotype [60, 61]. Although eye contact problems are usually not present during the first year of life, they evolve over time as do the sensory hyperarousal, anxiety, motor and social deficits. Hoeft et al [62] have reported that the early trajectory of brain growth abnormalities in FXS becomes more exaggerated over time and includes enhanced growth of the caudate, nucleus basalis, and thalamus, compared to controls. Those authors also documented enhanced white matter volume, particularly of the striatal-frontal regions, becoming more dramatic in the early years (1 to 3 years) and suggesting axonal pathology as opposed to secondary connectional dysregulation [62]. Their work further suggests that the earlier the intervention is begun, the better for an individual with FXS. These findings provide neurobiological support for initiating interventions as early in the lifespan as

possible, although further clinical studies are needed. A summary of treatment for FXS is reviewed by Hagerman et al [63].

RNA Toxicity and the Premutation Carrier

The discovery of the neurodegenerative disorder, FXTAS, in older adult carriers of premutation alleles, coupled with increased *FMR1* transcriptional activity in the premutation range, led to the recognition of an entirely distinct pathogenic mechanism associated with the *FMR1* gene, namely, RNA toxicity [64-68]. A range of studies of the adverse consequences of expressing the expanded CGG repeat in human, animal, and cell models, has helped to establish an RNA toxicity model involving a - toxic gain-of-function of the premutation CGG-repeat *FMR1* RNA [69-79]. However, though carriers of premutation alleles have elevated *FMR1* mRNA [27, 80, 81], the strongest argument for an RNA-based toxicity mechanism in FXTAS, as well as FXPOI [82-84], is that these clinical syndromes are limited to the premutation repeat range, where the gene is active; that is, low levels (or absence) of FMRP is not sufficient to cause either FXTAS or FXPOI. However, moderately lowered FMRP levels in the upper premutation range may compound the effects of elevated RNA levels – a mechanistic issue that still needs to be resolved – but the primary effect appears to be expression of the expanded CGG-repeat RNA. A supporting argument for an RNA-based mechanism is that the *FMR1* mRNA is found within the characteristic intranuclear neuronal and astrocytic inclusions of FXTAS [85, 86].

FXTAS was originally described as a late-adult-onset neurodegenerative disorder; however, there is an emerging view that FXTAS, and likely also FXPOI, is the end-stage of a process that actually begins in early development, and which may be responsible for the emotional and behavioral problems, cognitive impairment, ASD, and seizure activity experienced by children who are carriers of premutation alleles [21, 25, 87]. This view is based on a combination of animal- and cell-based studies for early abnormalities resulting from expression of the premutation allele. In particular, Chen et al. [43] demonstrated that in cultured hippocampal neurons from day one postnatal premutation (knock-in, KI) mice, there were CGG-repeat-dependent decreases in both the number of branches and the interbranch lengths, and decreased longevity in culture. Moreover, Garcia-Arocena et al. [88] observed abnormal lamin A/C architecture, with loss of ring-like nuclear staining, in embryonic fibroblasts from the KI mouse. In behavioral studies with the KI mice, there were

progressive deficits in spatial processing (but no motor involvement) in mice as young as 12 weeks [70, 89]. These observations, plus elevated levels of *FMR1* mRNA in children with premutation alleles [90], support the presence of an early developmental component of *FMR1* mRNA-associated toxicity.

Based on the toxic RNA gain-of-function model for myotonic dystrophy, in which disease pathogenesis involves the sequestration of one or more proteins by an expanded rCUG repeat in the 3' untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene [91, 92], the first view of FXTAS envisioned a similar, direct-RNA mechanism in which proteins would be sequestered by the expanded CGG repeat [19, 65, 67, 69]. A growing number of cell- and animal-based studies support this "direct RNA" model [71, 72, 93, 94]. Recently, Sellier et al. [94]presented evidence for both sequestration of an RNA processing protein, Sam68, and the consequent altered splice-site regulation of several RNAs whose splicing is known to be regulated by Sam68. In addition to their demonstration of the functional consequences of Sam68 sequestration, Sellier et al. demonstrated that the incorporation of the protein into nuclear aggregates displayed a CGG-repeat cutoff such that aggregation only occurred for expansions exceeding ~40 CGG repeats. More recently, Sellier et al [95] also reported that a consequence of this sequestration is dysregulation of microRNAs, which may be related to the clinical problems of premutation carriers.

It should be noted that while the sequestration model remains the most viable mechanism for RNA toxicity, the clinical data only support the requirement for transcription. Thus, one cannot discount a role for other mechanisms such as RNA-triggered signaling or co-transcriptional mechanisms [68] (Figure 3). Thus, evidence for a direct RNA-based (e.g., sequestration) model cannot exclude the possibility that co-transcriptional RNA, or even DNA, has a role in the pathogenesis. Entezam and Usdin [74] observed that the DNA-repair protein ATR is recruited to CGG-expansions, and the fact that another DNA-repair protein, γ-H2AX[96], is found in the intranuclear FXTAS inclusions [97], suggests that transcription-induced DNA damage could also trigger the pathogenesis of premutation-associated disorders.

Recent work from the laboratory of Guilivi has demonstrated mitochondrial dysfunction in fibroblasts and brain samples in premutation carriers both with and without FXTAS [44]. Mitochondrial dysfunction in carriers included uncoupling between electron transport and synthesis of ATP in addition to decreased levels of

mitochondrial proteins including the ATPase β-subunit (ATPB) from complex V, cytochrome c oxidase subunit IV from Complex IV (CCOIV) and MnSOD as part of the mitochondrial antioxidant defense. The levels of the mitochondrial proteins correlated inversely with the CGG repeat numbers in the premutation range. These protein changes increased oxidative stress, increased oxidatively modified mitochondrial proteins and activated the unfolded protein response (UPR) and phosphorylation of the alpha subunit of the heterotrimeric eukaryotic translational initiation factor 2 (eIF2α), resulting in a decrease in protein translation. Similar types of mitochondrial abnormalities have been seen in those with autism without a fragile X mutation [Giulivi et al unpublished data, 98, 99]. Specifically, Olivera et al [98] reported that 14 of 69 patients with autism had hyperlactacidemia and in 5 of 11 of these patients who underwent a deltoid muscle biopsy there was a mitochondrial respiratory chain disorder with enzyme function that was < 20% of normal mean activity including complex I, complex IV and complex V abnormalities. Weissman et al. studied 25 patients with autism and evidence of oxidative phosphorylation abnormalities and found 19 with elevated lactate levels and 64% with complex I deficiency, and 20% with complex III deficiency. Two of the patients had mt DNA pathological mutations [99]. Other reports of mitochondrial gene mutations in children with autism have been reported [100-102].

Clinical Involvement of Some Premutation Carriers

Although autism and other clinical involvement in a subgroup of young premutation carriers was initially thought to be only an occasional occurrence [26, 103-106], research cohorts demonstrated that approximately 14% of premutation boys and 5% in premutation girls had ASD [107]. More recent studies have demonstrated a high rate of ASD (73%) in premutation boys who are referred clinically to the UC Davis MIND Institute, although it is much lower in premutation males who are identified by cascade testing (7%) compared to brothers who do not have the premutation (0%) [108]. Although there is clearly a bias towards an ASD phenotype in those who present clinically, a recent on-line family questionnaire filled out by over 1200 families affected by FXS detected an autism diagnosis in 19% of 57 males with the premutation, which is significantly different than controls (5%), and in 1% of 199 females with the premutation [87]. This same survey found that 33% of premutation boys had developmental delays, significantly different from age matched

boys without the premutation (1.8%). A completely unbiased population of premutation carriers that should be followed carefully are those diagnosed with newborn screening and 3 studies are currently in progress in the US.

Studies of neuropsychological deficits in premutation carriers during adulthood have been complicated because of the subclinical CNS changes that can occur related to the development of FXTAS [109-111] Studies have detected deficits in executive function in a subgroup of males with the premutation, but not females [112-117]. In contrast to these 4 centers, Hunter et al [118] found no neuropsychological deficits in 54 premutation males who were under age 50, although they did not use the Behavioral Dyscontrol Scale (BDS) [113, 119], which was found to be most sensitive to executive dysfunction in older male carriers [112]. Clearly, recruitment bias is likely to affect the adult premutation studies in neuropsychological testing and in emotional assessments. In contrast to the neuropsychological testing, standardized emotional assessments demonstrating problems with anxiety and/or depression have been found in both males and females with the premutation both with and without FXTAS compared to controls at multiple centers [22, 120-124]

An emerging phenotype includes the finding of autoimmune problems in a subgroup of women with the premutation. These problems include fibromyalgia, hypothyroidism and multiple sclerosis and they can occur in premutation women both with and without FXTAS [24, 125-127]. Hunter et al [82] found that women with irregular cycles reported higher rates of thyroid disease in addition to depression/anxiety. The molecular process leading to the autoimmune problems are unknown although they are most likely related to the RNA toxicity. Predisposing factors leading to autoimmune disease in some females are likely genetic because they may cluster in families in our clinical experience. Because of concern for genetic factors that underlie both autoimmune disease and autism we studied whether there is an increase in ASD with FXS in the children of female carriers who have autoimmune disease compared to carriers who do not have autoimmune disease [128]. The odds ratio (OR) for ASD was 1.27 (p= 0.51) which was not significant, however, the OR for seizures and tics in the offspring were 3.81 (p=0.031) and 2.94 (p=0.019) respectively. These results raise the possibility that there are intergenerational autoimmune factors or perhaps auto-antibodies that affect the

prevalence of seizures and tics in the offspring of premutation mothers with autoimmune disease [128].

FMRP Function Throughout Life Leading to Targeted Treatments for FXS

FMRP is an mRNA-binding protein that is important for mRNA transport, mRNA stabilization, and translation of mRNA into protein at the synapse [129-131]. FMRP is also a factor in the regulation of adult neurogenesis, so in the absence of FMRP there is dysregulation of glycogen synthase kinase 3 β (GSK3β), reduced β-catenin, and defective Wnt signaling; these alterations lead to down-regulation of neurogenin 1, which is an early initiator of neuronal differentiation and an inhibitor of astrocyte differentiation [132]. Therefore FMRP is important throughout life and there is a high incidence of motor problems, including Parkinson's Disease (PD), with aging in those with FXS [133]. In addition, in neuropathologic studies there is evidence of migration problems in the hippocampus and in the cerebellum in those with FXS (Greco et al unpublished data) that is similar to the migration problems reported in individuals with autism [134]. These problems may be related to dysregulation of Wnt signaling in both FXS and autism.

Perhaps the most important change in protein expression in the absence of FMRP is the excess basal translation of proteins involved in the metabotropic glutamate receptor 5 receptor (mGluR5) pathway [135]. Bear and colleagues have proposed the mGluR theory of fragile X suggesting that the deficits associated with FXS are related to up-regulation of the down stream effectors of the mGluR5 pathway, leading to enhanced long term depression (LTD) and that treatment with an mGluR5 antagonist will be a targeted treatment for FXS [135, 136]. Both FMRP and mGluRs play important roles in synaptogenesis and synaptic plasticity and in the absence of FMRP there are long, thin and immature dendritic spines in both human and animal models of FXS [137-142]. There are also enhanced, abnormal epileptiform discharges consistent with an enhanced rate of clinical seizures in FXS [143, 144].

Support for the "mGluR theory" has been shown by generating *FMR1* mutant mice with a 50% reduction in mGluR5 expression [145]. The mGluR5 deficiency rescued most of the KO mouse abnormalities including altered ocular dominance plasticity, increased density of dendritic spines on cortical pyramidal neurons, increased basal protein synthesis in the hippocampus, exaggerated inhibitory

avoidance extinction, audiogenic seizures and accelerated body growth. However, macroorchisism was not rescued. This work is supportive of the Bear et al [146] proposal that excessive mGluR5 signaling is responsible for the psychiatric and neurological symptoms of FXS including cognitive deficits, seizures, anxiety, perseverative movements, and social deficits.

Use of mGluR5 antagonists in animal models of FXS further supports the mGluR theory. MPEP (2-methyl-6-phenylethynyl pyridine hydrochloride) is a potent, highly selective antagonist of mGluR5 receptors [147]. In vitro, both MPEP and fenobam, another mGluR5 antagonist, were able to rescue hippocampal dendritic abnormalities in the KO mice [148, 149]. MPEP has reversed audiogenic seizures, epileptiform discharges, open field hyperactivity, and the defect in prepulse inhibition (PPI) of startle in the KO mice [148-150]. When MPEP and lithium, a partial mGluR5 antagonist which also blocks GSK3B, were given to *dfmr1* loss-of-function *Drosophila* mutants, the flies had restored normal courtship behavior, memory, and brain structural abnormalities through the reduction of mGluR activity [151]. MPEP is toxic to humans so other mGluR5 antagonists have been studied including fenobam in FXS [152, 153]. Fenobam was found safe in a single dose trial in 12 adults with FXS. There were improvements in hyperactivity and anxiety and 50% showed at least a 20% improvement in PPI [152]. Currently there are 2 additional mGluR5 antagonists undergoing trials in adults with FXS at multiple centers [153].

Other mechanisms to down-regulate glutamate release and modulate mGluR overactivity have been investigated. $GABA_B$ receptor agonists, such as baclofen, inhibit both presynaptic release of glutamate and postsynaptic transmission and/or intracellular signaling downstream from mGluR5 [154-156]. Baclofen has been shown to be efficacious in treating hyperactivity [157], marble burying (Seaside Therapeutics, unpublished data), and audiogenic seizure phenotypes in fragile X KO mice [158]. A double-blind, placebo-controlled, crossover trial of Arbaclofen, the right sided isomer of baclofen that is significantly more potent that regular baclofen as a GABA agent, has just been completed at multiple centers in over 60 individuals with FXS (6 years and older). The preliminary safety and efficacy results are positive with improvement in the CGI-I in those with the most severe baseline ratings [159].There are also preliminary studies which are taking place with those with autism without FXS and these studies have preliminary positive results. Therefore further studies will take place in both FXS and in autism.

The GABAergic system is also dysregulated in FXS and GABA agents are important to consider for targeted treatment studies in FXS. GABA is a major inhibitory neurotransmitter receptor in the brain and important in anxiety, depression, epilepsy, insomnia, and learning and memory [160]. GABA-mediated inhibition is important for terminating ictal discharges and the spread of hyperexcitability, which can lead to seizures [161].

There are two main subtypes of GABA receptors: $GABA_A$ and $GABA_B$. The main difference between them is that the first is a ligand gated Cl^- channel that gives fast inhibition, while the latter is a G-protein coupled receptor which gives slow and more prolonged inhibitory signals [162, 163]. The metabotropic $GABA_B$ receptor can either be presynaptic and inhibit the release of neurotransmitters through down-regulation of high-voltage activated Ca^{2+}-channels; or, when postsynaptic, decrease neuronal excitability through its influence on K^+ channels. Thus, $GABA_B$ agonists such as Arbaclofen mediate their down regulating effects on either side of the synapse. The ionotropic $GABA_A$ receptor is usually localized postsynaptically and their activation leads to opening of Cl^- channels and hyperpolarization of the membrane potential, thus making it difficult for excitatory neurotransmitters such as glutamate to generate an action potential. $GABA_A$ receptors are more abundant than $GABA_B$ receptors in mammalian brain, and disorders such as epilepsy, sleep disorders and anxiety are now being treated using drugs that act on the $GABA_A$ receptor.[164].

Direct binding between FMRP and the mRNA of the delta subunit of the $GABA_A$ receptor has been shown [165]. Reduced expression and dysfunction of several subunits of the $GABA_A$ receptor (α1, α3, α4; β1, β2; γ1, γ2, and δ) have been shown in fragile X animal models [166-168]. *FMR1 Drosophila* mutants destined to die from glutamate toxicity were rescued after administering molecules involved in the GABAergic pathway [166]. In addition, abnormal male courtship behavior and mushroom body abnormalities were rescued by GABA agents [166]..

There is a profound reorganization of neocortical inhibitory circuits of GABAergic intraneurons in the KO mouse [164, 167-173]. Recent evidence indicates

that deficits in GABA-mediated inhibition may underlie many of the key symptoms in FXS, including the seizures, anxiety and autistic-like behaviors [167, 169, 173]. The neocortex in the KO mice exhibits a marked reduction in the density of parvalbumin-staining GABAergic interneurons. Moreover, electrophysiological studies in brain slices from these animals exhibit impaired $GABA_A$ receptor-mediated inhibitory function [174]. In addition to a gross reduction in GABA-mediated inhibition caused by the maldevelopment of inhibitory circuits and the loss of GABAergic interneurons, there is also evidence of altered $GABA_A$ receptor subunit expression in the fragile X KO mouse [167]. In particular, there appears to be a selective reduction in the expression of δ subunits [167, 168]. Global expression analysis by means of the differential display in the fragile X mouse model revealed consistent underexpression of only 3 genes; one of these was the $GABA_A$ receptor subunit δ. As $GABA_A$ receptors are the major inhibitory receptors in the brain and are specifically involved in processes that are disturbed in fragile X, including neuronal excitability (leading to enhanced seizure susceptibility), anxiety, sleep and learning, enhancement of the function of $GABA_A$ receptors may have major therapeutic benefits for FXS. Kooy and colleagues [175] have demonstrated that use of a $GABA_A$ agonist ganaxolone improved seizures in the KO mouse model of FXS. Ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a 3β-methylated synthetic analog of the progesterone metabolite allopregnanolone, which is itself a neuroactive steroid. Unlike progesterone, neither allopregnanolone nor ganaxolone have direct hormonal activity via progesterone receptor activation and cannot cause hormonal side-effects. However, allopregnanolone and ganaxolone are powerful positive allosteric modulators of $GABA_A$ receptors [161]. Human trials indicate that ganaxolone is well tolerated and that it may be efficacious in the treatment of diverse forms of epilepsy in children and adults [176-180]. Plans for studies of ganaxolone are currently underway for children and adults with FXS.

Minocycline, a widely used antibiotic used to treat acne and skin infections, is another promising drug that may target core symptoms of FXS and autism. Minocycline inhibits matrix metalloproteinase-9 (MMP-9) and reduces inflammation in the central nervous system. Matrix metalloproteinases are enzymes involved in synaptic plasticity and are associated with immature dendritic spine morphology [140, 181]; MMP-9 is elevated in FXS. When minocycline was administered to *FMR1*

KO mice, their hippocampal neurons exhibited mature dendritic spines and behaviorally, they showed decreased anxiety and improved exploration skills [140]. Off-label use of minocycline to treat 50 individuals with FXS resulted in two-thirds of families noticing positive improvements in their child's language, attention, and/or behavioral improvements while on the medication [182]. An open label trial is ongoing to investigate the effects of minocycline on children with regressive autism at NIMH. Paribello reported beneficial effects on the CGI and the Aberrant Behavior checklist in those 13 and older with FXS treated in an open trial of minocycline [183]. Currently a double-blind, placebo-controlled clinical trial is in progress for individuals with FXS from the ages of 3.5 to 16 years at the MIND Institute.

FXS has led the way for targeted treatments in neurodevelopmental disorders, and many of the treatments guided by molecular abnormalities in FXS may also be helpful for non-fragile X autism. The treatment trials will now combine targeted treatments, which strengthen synaptic connections, with enhanced educational and behavioral interventions to further develop appropriate synaptic connections in FXS. These targeted treatments combined with educational interventions look promising for reversing the intellectual and behavioral problems of FXS. Due to shared neurobiological and molecular pathways, these interventions will hopefully also prove helpful in a subset of patients with idiopathic autism

Conclusions

Fragile X syndrome and autism are intertwined because FMRP regulates the translation of many messages that affect synaptic plasticity and connectivity in the central nervous system. The absence of FMRP also leads to up-regulation of mGluR5 pathways and down-regulation of $GABA_A$ pathways and targeted treatments to reverse these problems are currently being studied in patients with FXS. Many of these targeted treatments may also be helpful for ASD without FXS.

The premutation can also cause ASD, particularly in a subset of young males and the mechanism of involvement relates to elevated mRNA levels causing dysregulation of numerous proteins, early neuronal cell death in culture, mitochondrial dysfunction, and vulnerability to environmental toxicity. Targeted treatments are currently being developed for premutation involvement in early

childhood and also for neurodegenerative problems including FXTAS in aging individuals.

Competing Interests

Dr. Randi Hagerman has received funding from Seaside Therapeutics, Novartis, Roche, Forest, Johnson & Johnson, and Curemark for clinical trials. She also consults with Novartis and Roche regarding clinical trials in fragile X syndrome. Dr. Paul Hagerman is an unpaid consultant with Asuragen, and has a filed patent application for an *FMR1* genotyping method. Gry Hoem has no conflicts of interest.

Author's Contributions

All authors helped draft the manuscript, and all authors read and approved the final manuscript.

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Figures

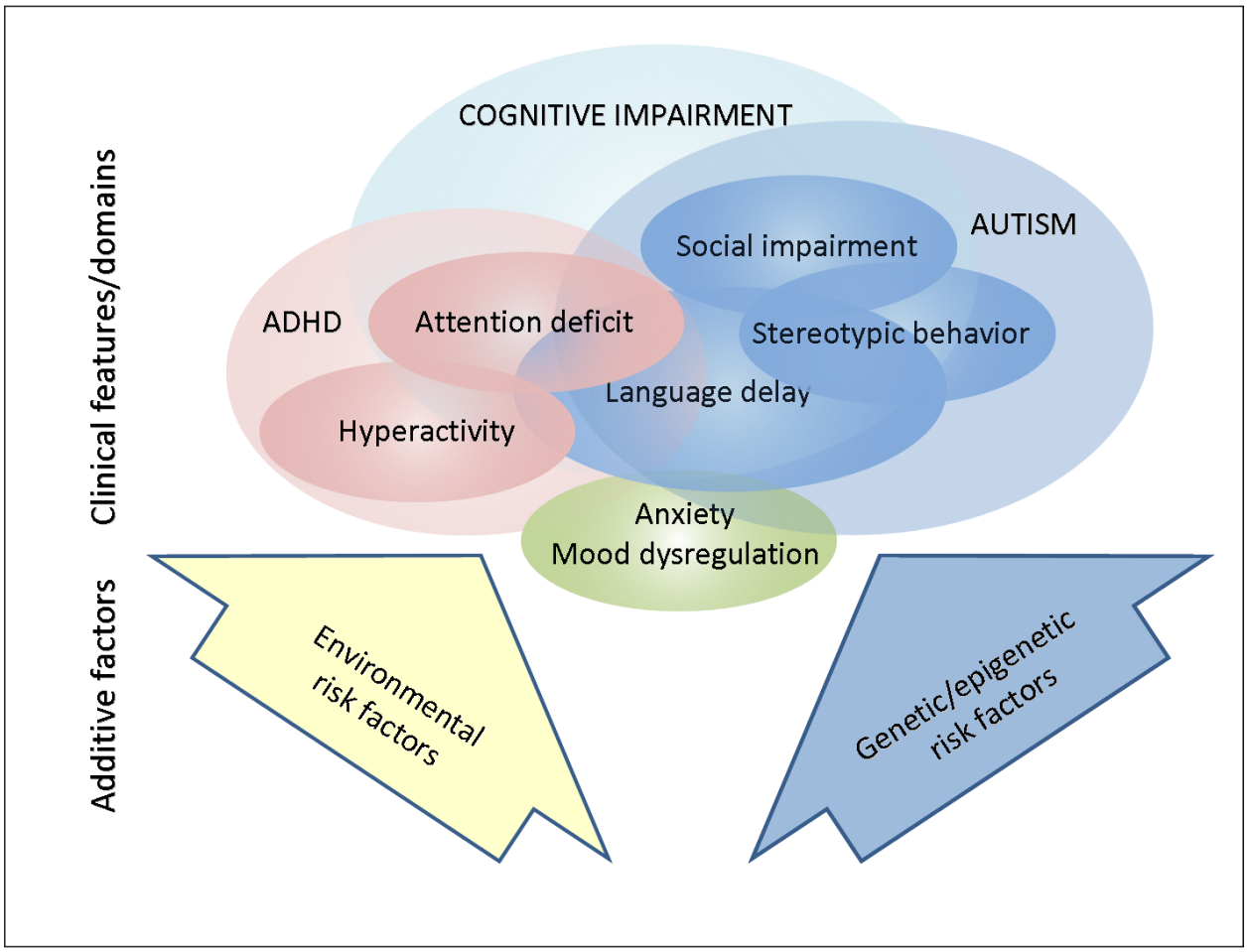

Figure 1 – Overview of the Behavioral/Cognitive Phenotype of Fragile X Syndrome.

The interrelationships among cognitive, behavioral and attentional deficits in fragile X syndrome are modified by additional environmental influences and genetic background effects. Environmental influences include seizures, trauma, abuse, and socioeconomic status. Genetic influences include allelic variations, additional genetic disorders and variation in the expression levels of genes important for the phenotype of FXS.

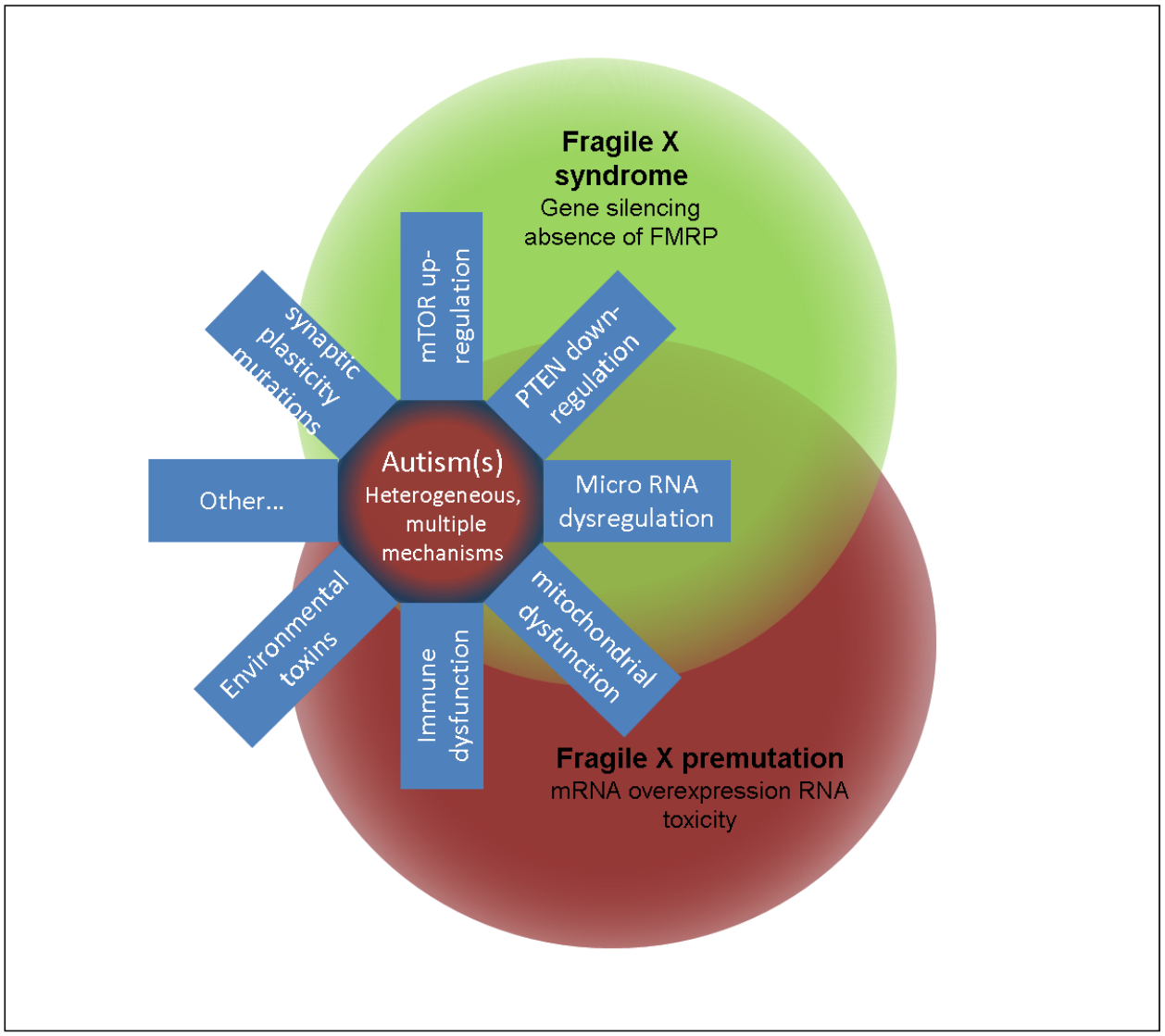

Figure 2 – Molecular Overlap Among Autism, Fragile X Syndrome and Premutation Disorders.

The absence of FMRP leads to the dysregulation of a number of proteins including those involved with synapse formation and plasticity, glutamate and GABA neurotransmission, and mTOR and PTEN pathways. The premutation is associated with elevation of *FMR1*-mRNA leading to sequestration of proteins and mitochondrial dysfunction. Many of these some molecular changes can also occur in some types of autism. Some patients with fragile X syndrome have mosaicism of premutation and full cells so there is overlap of the molecular mechanisms among all three disorders.

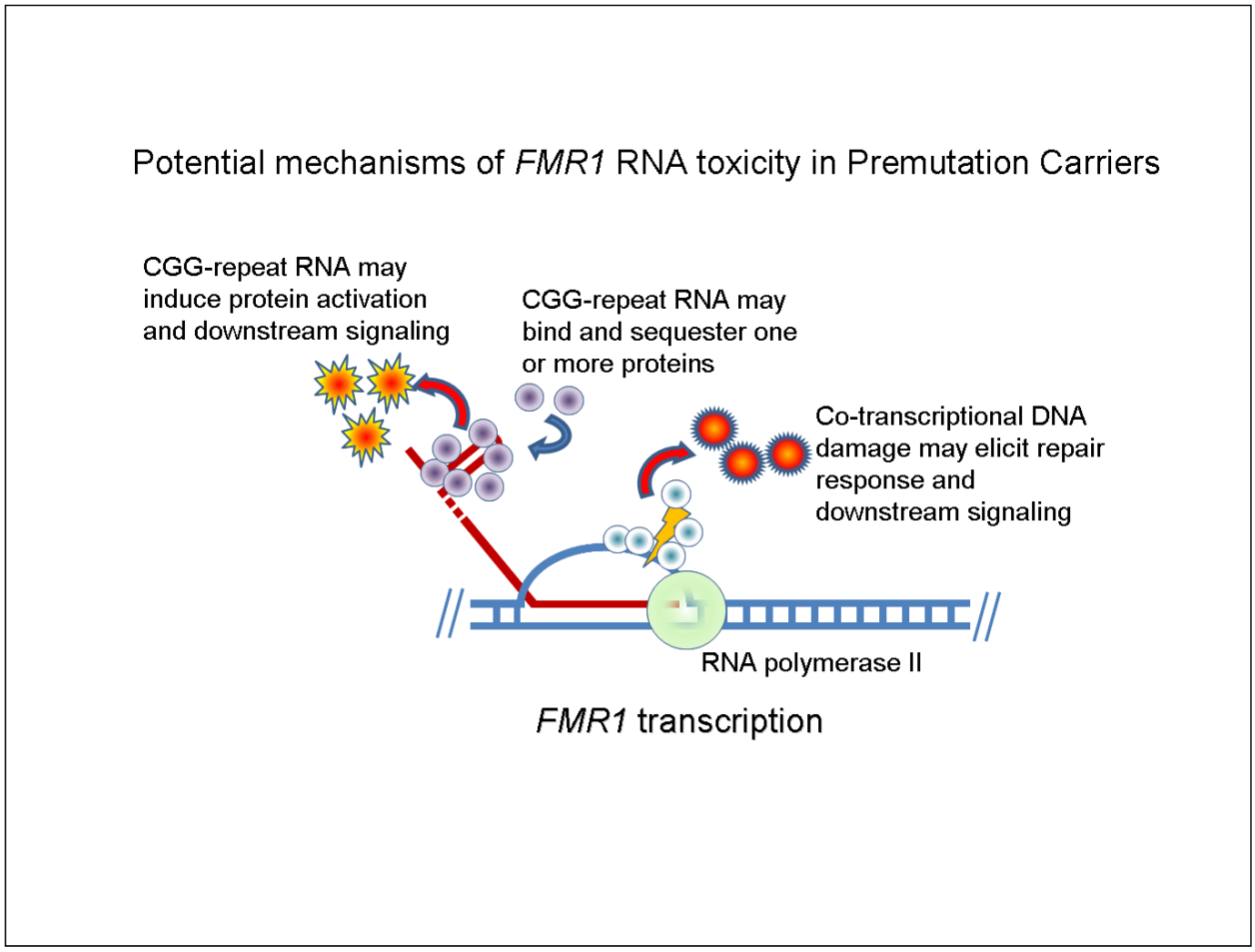

Figure 3 – Potential mechanisms of *FMR1* mRNA toxicity

Although numerous studies point to RNA toxicity as the underlying pathogenic trigger in FXTAS, the specific mechanism for such toxicity is not known. Possibilities include (i) sequestration of one or more proteins that bind to the RNA, thus attenuating their other cell functions; (ii) protein activation upon binding to the CGG-repeat RNA, leading to dysregulation of one or more signaling cascades; (iii) various co-transcriptional process, such as R-loop formation, that lead to DNA damage/repair signaling and consequent cellular dysregulation.