

Review Article

Lessons from Fragile X Regarding Neurobiology, Autism, and Neurodegeneration

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ABSTRACT. The fragile X mental retardation 1 gene (*FMR1*) mutation causes two disorders: fragile X syndrome (FXS) in those with the full mutation and the fragile X-associated tremor/ataxia syndrome (FXTAS) in some older individuals with the premutation. FXS is caused by a deficiency of the *FMR1* protein (FMRP) leading to dysregulation of many genes that create a phenotype with ADHD, anxiety, and autism. FXTAS is caused by the elevation of *FMR1*-mRNA to levels 2 to 8 times normal in the premutation. This causes an RNA gain of function toxicity leading to brain atrophy, white matter disease, neuronal and astrocytic inclusion formation, and subsequent ataxia, intention tremor, peripheral neuropathy, and cognitive decline. The neurobiology and pathophysiology of FXS and FXTAS are described in detail. *J Dev Behav Pediatr* 27:63–74, 2006. Index terms: *Fragile X, neurobiology, autism, neurodegeneration.*

This review highlights the advances in the fragile X field in the last five years. There have been remarkable discoveries in the molecular biology of the fragile X mental retardation 1 gene (*FMR1*) which sets the stage for considering fragile X syndrome (FXS) as a portal disorder for understanding many other complex genetic conditions including autism, attention deficit hyperactivity disorder (ADHD), anxiety, mood instability, and epilepsy. The emergence of a new phenotype in older premutation carriers, the fragile X-associated tremor/ataxia syndrome (FXTAS), has led to the discovery of a new mechanism of disease from the *FMR1* mutation.¹ This review will discuss clinical involvement of autism in FXS and neurodegeneration in FXTAS in light of the new information concerning molecular mechanisms and neurobiological advances. The genetic mechanisms affecting clinical involvement in the full mutation and the premutation will enhance the treatment and genetic counseling efforts of clinicians and influence the concepts of researchers who are investigating complex genetic disorders.

To review basic concepts, there is a repetitive CGG sequence on the front end of the *FMR1* gene and normal individuals have approximately 5 to 44 CGG repeats. There is a gray zone of 45 to 54 repeats that can be associated with minor instability from generation to generation. Research regarding clinical involvement and the possibility

of FMRP deficiency in the gray zone is taking place.^{2,3} The premutation is defined as 55 to 200 repeats by the American College of Medical Genetics³ and although most individuals with the premutation have a normal IQ, premature ovarian failure (POF) occurs in approximately 21% of female carriers⁴ and FXTAS occurs in approximately 38% of older male carriers.⁵ Some individuals with the premutation have lower *FMR1* protein (FMRP) levels, in addition to features of FXS.^{1,6–8} All individuals with the premutation have elevated *FMR1*-mRNA^{1,6,7} that is described in more detail below. The full mutation of the *FMR1* gene involves an expansion of >200 CGG repeats and it is clinically associated with features of FXS including prominent ears, long face, hyperextensible finger joints and cognitive deficits including learning disabilities or mental retardation.^{9,10} Usually the full mutation expansion is methylated and transcription of the gene is significantly reduced or eliminated so there is little or no *FMR1*-mRNA.¹¹

The prevalence of FXS with the full mutation and mental retardation (MR) is approximately 1 per 4000 males and 1 per 6000 females.^{12,13} However, the spectrum of involvement in fragile X is broad, including learning disabilities, emotional problems, and/or mental retardation. The prevalence of the premutation in the general population is approximately 1 per 259 in females,¹⁴ although in Israel it is higher, approaching 1 per 113.^{15,16} In males the prevalence of the premutation is approximately 1 per 810.¹⁷ When we include the clinical involvement in some individuals with the premutation in addition to the broad spectrum of involvement in FXS, the prevalence of clinical problems from the *FMR1* mutation is substantially higher than 1 in 4000.

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MOLECULAR ASPECTS OF THE FRAGILE X PHENOTYPE

We now know that the *FMR1* protein (FMRP) is an RNA binding protein that is involved with the regulation of translation of many other messages in the neuron.¹⁸⁻²⁰ In those with FXS and the full mutation FMRP is absent or deficient so the messages that FMRP binds to become dysregulated. FMRP typically functions as a translation suppressor that is involved in synaptic plasticity through regulating local protein synthesis of specific mRNAs in response to synaptic stimulation.^{21,22} There is evidence that FMRP regulates translation through an inhibitory microRNA pathway that also involves Dicer, a protein that cuts RNA into small pieces leading to RNA interference and inhibition of translation.^{23,24} FMRP binds to messages either at the synapse or in the nucleus and then it subsequently transports these messages to the synapse on a kinesin motor.^{25,26} With stimulation of the metabotropic glutamate system, FMRP organizes the translation of messages important for synaptic structural changes or synaptic plasticity.^{22,26,27}

The *FMR1* mutation not only affects the gene product, FMRP, but many other genes. A genomics perspective, one that involves multiple genes throughout the human genome, now dominates our understanding of the FXS phenotype because the absence of FMRP dysregulates many genes whose translation is normally regulated by FMRP. A number of messages which are regulated by FMRP have now been identified.^{19,20,28,29} Anxiety which is a common feature of fragile X may be related to the dysregulation of the glucocorticoid receptor whose message binds to FMRP.²⁰ Epilepsy which occurs in 20% of individuals with fragile X may be related to the dysregulation of the Gamma-aminobutyric acid_a (GABA_a) receptor whose message binds to FMRP. There are a number of mRNAs, including those of the cadedrins and MAP1B, that are important for synaptic plasticity and synaptic structure and their dysregulation likely adds to the mental retardation (MR) phenotype of FXS.^{18-20,28}

An important pathway for cognitive development is the metabotropic glutamate receptor 5 (mGluR5) pathway (a member of group I metabotropic glutamate receptors) which leads to long term depression (LTD), weakened synaptic connections and eventually synaptic elimination. LTD is important in normal brain development so that unstimulated synaptic connections can be eliminated. Through long term potentiation (LTP) other synaptic connections are made stronger with stimulation. The mGluR5 pathway requires protein synthesis and FMRP normally inhibits this protein synthesis, so FMRP puts the brakes on LTD through the mGluR5 pathway. However, in the absence of FMRP as in FXS, LTD is dramatically enhanced leading to weak, immature and elongated synaptic connections.^{30,31} Enhanced LTD was first discovered in fragile X knock-out mice, that have an *FMR1* gene that is partially or completely eliminated^{30,31} and has been documented in the cerebellum in the humans with FXS,³² and has been treated in the fragile X *Drosophila* model.³³ The enhanced mGluR5 pathway in FXS also leads to

internalization and therefore reduction at the synapse of alpha-amino-5-methyl isoxazole-4-propionate (AMPA) receptors particularly in the hippocampus. A number of clinical features including epilepsy, MR, hypersensitivity to tactile stimuli, social deficits, and even loose stools have been hypothesized to be related to enhanced mGluR5 activity and LTD in FXS.³¹ This is important for clinicians to understand because these findings have direct therapeutic implications. Both mGluR5 antagonists and ampakines that stimulate the AMPA receptors are in investigational stages of development and they have potential to be specific treatments for FXS in the future.

A Broad Spectrum of Clinical Involvement

Our understanding of clinical involvement in fragile X has broadened to include not only mental retardation (MR), but also learning disabilities and psychiatric problems. These additional problems are often seen clinically as outlined in the family pedigree in Figure 1. The most severely involved patients with FXS often have autism and are nonverbal.^{9,34} Mild involvement without MR occurs when FMRP is only mildly deficient.^{35,36} This occurs in high functioning males who have a full mutation that is unmethylated, in those who have a full mutation and a high percentage of cells with the premutation (mosaic), or in females who have a favorable activation ratio; specifically the majority of their cells have the normal X as the active X.^{9,35-37} In studies that include a large number of patients with FXS, there is a significant relationship between IQ and level of FMRP.^{35,36,38} In studies that do not include a number of high functioning individuals, the relationship between FMRP and IQ is less significant.³⁹ Approximately 15% of males and 70% of females with FXS have an intelligence quotient (IQ) greater than or equal to 70.^{10,35,38,40,41} Many females and some males may present with psychiatric diagnoses such as generalized anxiety disorder, social phobia, selective mutism, or Asperger syndrome without MR.^{9,42-45}

An emerging new group of individuals involved with fragile X includes those with the premutation (55 to 200 CGG repeats) who may present with ADHD, learning disabilities, MR, or autism spectrum disorder (ASD).^{7,8,46} Sometimes individuals with the premutation have a lower level of FMRP compared to normals which can lead to features of FXS, such as poor eye contact, hand flapping, hand biting, perseverative speech, sensory hyperarousal, and anxiety.^{6,7} There is a relative block in translation leading to lowered FMRP levels and higher *FMR1*-mRNA levels in the premutation range.⁴⁷⁻⁴⁹ This block in translation is likely related to the CGG expansion in the *FMR1*-mRNA that interferes with translation efficiency, although there is a difference in the start site for translation in the normal message *versus* the premutation message.⁴⁷

Although most premutation carriers have relatively normal levels of FMRP, some do not. If we define FXS as a syndrome of physical, cognitive, and behavioral features related to deficient levels of FMRP, it is possible to have FXS from the premutation because some individuals have

lowered FMRP and many features of FXS.^{6,8,46,50,51} This is an important concept for clinicians to understand because children with the premutation who present with mental retardation and features of FXS are often ignored and told that their problems cannot be due to the premutation. There are several case reports that have demonstrated low FMRP levels in individuals with the premutation who have features of fragile X.⁶⁻⁸ In addition to the symptoms of FXS that can be caused by lowered protein, there is an additional problem of elevated mRNA in the premutation that can also be associated with brain dysfunction as described below.^{1,49}

cytogenetic studies with FISH testing for 15q duplications/deletions and subtelomeric deletions, and *FMR1* DNA studies, demonstrates medical abnormalities in approximately 25%.⁵³⁻⁵⁹ *FMR1*-DNA testing is warranted in the evaluation of the child with autism of unknown etiology because 2 to 6% of these children will have the fragile X mutation.^{9,55-59}

The development and routine use of gold standard diagnostic tools for autism including the Autism Diagnostic Observation Schedule [ADOS⁶⁰] and Autism Diagnostic Interview [ADI⁶¹] have expanded our appreciation of social deficits and also increased the number of patients who are diagnosed with ASD in the fragile X population.³⁴ The association of autism and FXS was first recognized by Brown, Jenkins et al.⁶² in the early 1980s and within that decade approximately 15% of males with FXS were diagnosed with autism.^{63,64} In the 1990's these numbers were increasing to approximately 25%^{65,66} with the utilization of the Childhood Autism Rating Scale.⁶⁷ As the ADOS and ADI are more consistently used, the percentage

AUTISM

Autism is a behavioral diagnosis outlined in the DSM-IV,⁵² but the etiology is heterogeneous. Genetic studies have shown a high heritability and a careful medical evaluation, including an examination highlighting neurocutaneous signs and dysmorphic features, high resolution

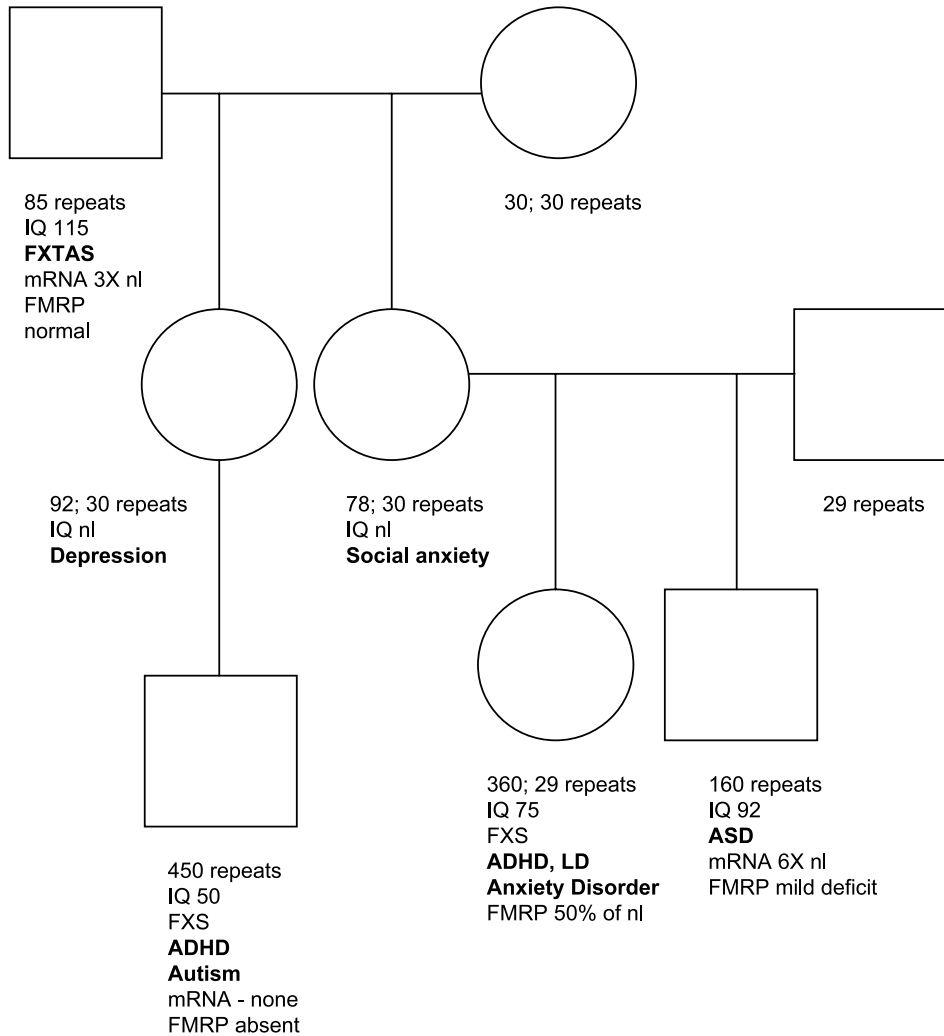


FIGURE 1. Pedigree of a family with fragile X demonstrating a spectrum of involvement including learning disabilities (LD), autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD). CGG repeat numbers in the *FMR1* gene are written below the circles for females and squares for males. nl = normal.

of patients with FXS who have autism has increased to 30% to 35%.^{34,68-70}

Research that has compared those with FXS and autism to those with FXS alone has demonstrated more severe cognitive, adaptive, receptive and expressive language deficits in those with both FXS and autism.^{34,69,71-73} Although the level of FMRP correlates with IQ,^{35,36,74} the level of FMRP does not predict the presence or absence of autism.^{75,76} This suggests that additional genetic factors have contributed to the sub grouping of children with FXS who also have autism as suggested by Rogers et al.³⁴ & Denmark et al.⁷³ In my experience patients with FXS who have known additional genetic conditions, such as Down syndrome or additional brain trauma, such as cerebral palsy or recurrent seizures, autism is typically present. However, in most cases with autism and FXS the possible additional genetic hits have not been identified. FXS is a unique population to study the

additional genes associated with autism because so many messages bind to FMRP. Since patients with FXS do not have FMRP they can reveal which additional genes are dysregulated in the autism phenotype. Microarray studies that assess the expression of thousands of genes simultaneously can be utilized in comparing children with FXS and autism to children with FXS without autism. Such studies should help to identify the genes whose translation is regulated by FMRP and may be additive to the autism phenotype.⁷⁷

Previous research has shown that environmental problems, such as low socioeconomic status or a poor home environment are associated with adaptive and cognitive deficits in FXS⁷⁸⁻⁸⁰ in addition to features of autism.⁷⁹ Hessler et al.⁷⁹ found that increased autistic behavior is associated with decreases in the quality of the home environment in males with FXS. In addition, children with FXS whose fathers refused to participate in the research

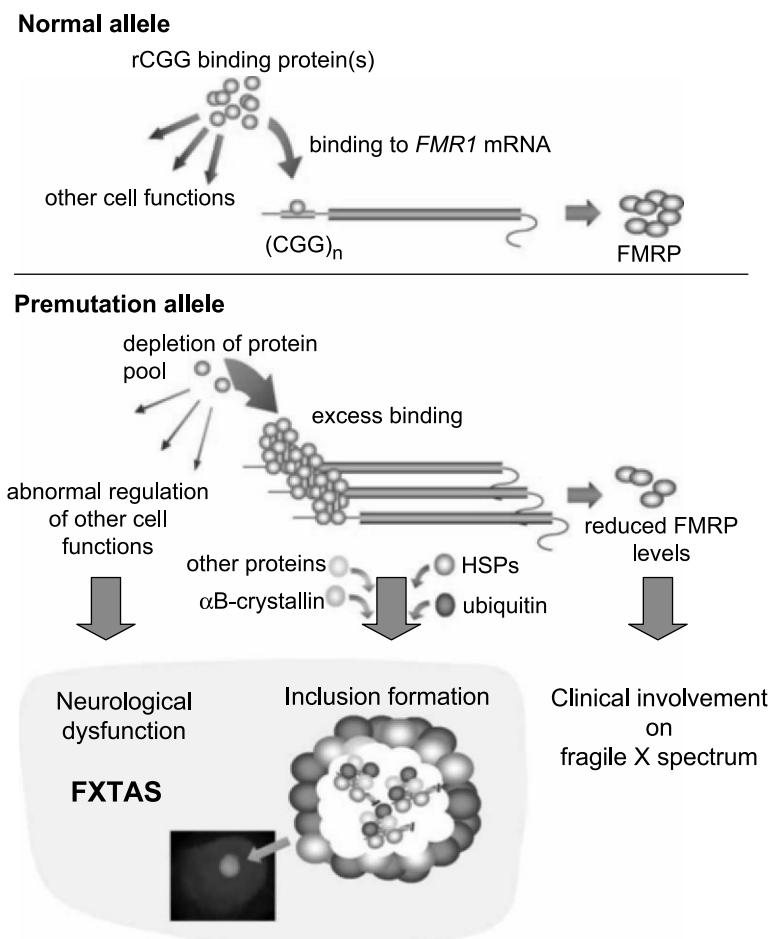


FIGURE 2. Figure modified from Hagerman and Hagerman 2004. Schematic representation of both mechanisms of involvement in the premutation including a mild deficit of FMRP leading to clinical features of fragile X and the RNA toxic gain-of-function model for FXTAS. In this model, specific protein interactions with the 5 UTR of the *FMR1* mRNA are altered as a consequence of expansion of the CGG repeat. In the premutation range, the expanded CGG repeat would lead to excess binding of one or more proteins, owing to increased mRNA copy number, increased number of CGG repeats, and/or altered secondary/tertiary RNA structure. This excess binding depletes the proteins from the cellular pool, resulting in the loss of their normal functions in other regulatory processes. The sequestration process would also trigger the accumulation or abnormal processing of proteins by the proteasomal degradation pathway, leading to inclusion formation with associated ubiquitinated proteins, proteasomal subunits, and stress-response (HSP) proteins. *Image inset.* Inclusion within an isolated neural cell nucleus (frontal cortex) from a patient with FXTAS; the inclusion is stained with fluorescent antiubiquitin antibody, and the nucleus is counterstained with DAPI.

or were unavailable for study were more likely to be autistic.⁷⁹ Although the problems in the parents who create a poor home environment may have a genetic or environmental basis, the mixture of both of these factors impacts the existence of autism in FXS. An important message for clinicians is that effective educational and therapeutic services also have a positive influence on behavior in FXS including autism.^{79,80}

An interesting subgroup of patients with FXS who usually have autism includes those who have the Prader-Willi (PW) phenotype.^{81–86} These individuals have symptoms of PW including severe hyperphagia, obesity, lack of satiation, and hypogenitalia, including a small penis but they eventually develop macroorchidism.⁹ The majority of those with FXS and the Prader-Willi phenotype also have autism, suggesting a second genetic hit which further disturbs hypothalamic function (Nowicki, Ferranti, Goodlin-Jones et al. in preparation). These individuals do not have a 15q deletion nor uniparental disomy, but the Prader-Willi phenotype in fragile X may come from dysregulation of CYFIP1 (cytoplasmic *FMRI* interacting protein) which works closely with FMRP.^{21,87} CYFIP1 was recently sequenced and localized to the 15q deletion region of Prader-Willi.⁸⁸ CYFIP1 also interacts with Rac1, a small GTPase which is important in cognitive functions.⁸⁷ It is likely that patients with the Prader-Willi sub phenotype of FXS also have dysfunction in CYFIP1. This maybe the first example of an additional gene related to FMRP that becomes dysregulated in FXS and autism. This phenotype is also one example of the heterogeneity that occurs within the fragile X and autism population. In our experience there are also variations in the severity of social deficits, anxiety, and hyperarousal to sensory stimuli in those with FXS and autism.

Those with FXS and autism are an important subgroup of patients with autism because they provide insight into the biological mechanisms that underlie other forms of autism. There will likely be commonalities in underlying molecular pathways for many of the known genetic causes of autism. There are several interesting neuro-anatomical and neurobiological similarities between FXS and autism without FXS. There is an increase in the head circumference in both fragile X^{89,90} and in autism without FXS.⁹¹ In young children with FXS and autism the head circumference is larger than with FXS alone [⁹²Chiu, Jenkins, Day et al. in preparation]. In FXS the posterior cerebellar vermis is smaller than controls^{93–95} and this is also found in some studies of autism.^{96,97} There is Purkinje cell drop out in both those with FXS and in those with autism,^{98,99} although this is seen in other disorders and may be nonspecific. AMPA receptors are decreased in FXS³¹ and in those with autism.¹⁰⁰ Early white matter tract alterations have been documented in children with FXS by diffusion tensor imaging, demonstrating disarray of the tracts.¹⁰¹ Most recently functional magnetic resonance imaging (fMRI) studies in idiopathic autism have demonstrated impaired connectivity and white matter abnormalities.^{102,103} Functional MRI studies in FXS have demonstrated a lack of normal recruitment of the neural network to solve math problems or carry out

executive function tasks.^{104–106} This functional finding is likely related to the elongated and immature dendritic spines which make weak and immature synaptic connections seen in neuropathological studies of human fragile X brains.¹⁰⁷ These findings are hypothesized to be related to enhanced LTD through mGluR5 pathways when FMRP is absent.³¹

Individuals with FXS have enhanced autonomic reactivity to sensory stimuli^{108,109} and a decrease in prepulse inhibition (PPI) suggesting problems with frontal gating.¹¹⁰ Similarly children with autism spectrum disorder (ASD) also have abnormalities in PPI which impact how they process sensory information.^{92,111} The remarkable similarities between FXS with autism and autism without FXS are likely related to the neurobiological consequences of the genetic overlap between these disorders.

An important new group of children with the *FMRI* mutation and autism are a subgroup of individuals with the premutation. Several reports have demonstrated MR or learning disabilities and autism particularly in some males with the premutation^{6–8,112} and further research is warranted.

INVOLVEMENT IN PREMUTATION CARRIERS AND FXTAS

Studies carried out in female premutation carriers have demonstrated emotional problems including anxiety, social phobia, and depression in approximately 30%,^{50,113–115} although cognitive deficits have not been detected in group studies.^{116,117} Some aspects of this emotional phenotype including withdrawn behavior and emotional sensitivity are more likely to occur in carrier females with >100 CGG repeats compared to those with <100 repeats,¹¹⁸ suggesting that it may be due to deficits of FMRP that occur in the upper premutation range.^{2,6,49} These symptoms appear to be a mild version of the more severe emotional problems including anxiety and mood instability that occur in those with the full mutation in both males and females.^{9,43,119,120} However, the emergence of a unique phenotype in females with the premutation which was not seen in those with the full mutation, specifically premature ovarian failure (POF) in approximately 20 to 25%,^{4,121,122} suggested that there were molecular abnormalities in the premutation that were not seen in the full mutation. The discovery of elevated *FMRI*-mRNA in males and females with the premutation confirmed this unique molecular manifestation of the premutation.^{2,6,49} Following on the heels of this finding was the discovery of FXTAS in older male carriers of the premutation.¹²³

Mothers of children with FXS were concerned about late onset neurological problems that their fathers with the premutation were experiencing and on further evaluation all of these grandfathers had a similar neurological phenotype of progressive intention tremor and/or ataxia beginning after age 50. In addition many of these patients had a peripheral neuropathy with decreased sensation to pinprick and vibration in the distal lower extremities, Parkinsonian features including masked facies, increased tone or difficulty initiating movement, psychiatric symptoms including anxiety,

Table 1. Diagnostic Criteria for FXTAS (adapted from Hagerman & Hagerman 2004 and Jacquemont et al. 2003)

Clinical Major	Clinical Minor	
• Intention tremor	• Parkinsonism	
• Gait ataxia	• Short-term memory deficit	
	• Executive function deficit	
Radiological Major	Radiological Minor	
• Symmetric white-matter lesions involving the middle cerebellar peduncles	• White matter lesions in cerebrum	
	• Moderate-to-severe generalized atrophy	
Definite:	Probable:	Possible:
• One clinical major criterion	• Two clinical major criteria	• One clinical major criterion
	<i>or</i>	• One radiological minor criterion
• One radiological major criterion	• One radiological major criterion	
<i>or</i>	• One clinical minor criterion	
• Presence of FXTAS inclusions on the basis of examination of post-mortem brain tissue		

Must have *FMR1* allele size of 55–200 CGG repeats.

irritability, or dysinhibition, autonomic dysfunction including impotence, and cognitive changes including memory and executive function deficits.^{124–128} Grandfathers with FXTAS did not demonstrate cognitive deficits in childhood and typically had higher education including graduate work. Many had long term anxiety or obsessive features in their personality which may have driven them to higher education levels coupled with high verbal comprehension scores that were significantly higher than controls.^{46,124} Adult male premutation carriers have been shown to have attention switching problems including a preference for fixed routines and a tendency to focus on details which may relate to their tenacity in educational achievement.¹²⁹ Recently Hessel, Tassone, et al.¹³⁰ have found in males with the premutation a significant correlation between elevated *FMR1*-mRNA levels and obsessive-compulsive symptoms on the SCL-90, an adult psychiatric rating scale. A similar correlation was seen in females who had an unfavorable activation ratio (AR < 0.5) meaning that the majority of their lymphocytes had the mutated X as the active X.¹³⁰

The radiological features of FXTAS include brain atrophy involving the cerebrum, brain stem and cerebellum with white matter disease in the periventricular, sub cortical and middle cerebellar peduncle (MCP) regions bilaterally, best seen with T2 weighted or flair imaging.^{124,131} Neuro-pathological studies of postmortem FXTAS brains demonstrate spongiosis with demyelination where the white matter disease occurs, Purkinje cell loss in the cerebellum and the presence of eosinophilic intranuclear inclusions in neurons and astrocytes throughout the cortex but not in Purkinje cells.^{132,133} The inclusions are most dense in the hippocampus and the inclusions have ubiquitin, heat shock proteins, *FMR1*-mRNA, myelin basic protein, α B crystallin and other components.^{132,133} Most importantly the inclusions do not have tau or α synuclein so they are different from the inclusions in tauopathies such as Pick's disease or the synucleinopathies, such as Lewy body dementias or Parkinson's disease (PD). Often individuals with FXTAS are misdiagnosed with PD or essential tremor or cerebellar ataxias¹³⁴ because they were not initially tested for the *FMR1* mutation. Recent screening studies

have shown that approximately 4% of adults presenting with cerebellar ataxia have the premutation.^{135,136}

The excess *FMR1*-mRNA in premutation carriers is hypothesized to exert a toxic gain of function effect leading to excessive binding of cellular proteins to the expanded CGG sequence and subsequent sequestration of these proteins and the mRNA in the inclusions (see Figure 2). The inclusions themselves may not be toxic but the sequestration of important proteins may lead to neuronal toxicity and cell death or apoptosis.¹³⁷ A similar mechanism of RNA toxicity occurs in myotonic dystrophy leading to sequestration of CUG binding proteins.¹³⁸ Support for the RNA toxicity model is found in the mouse work of Willemsen, Hoogeveen-Westerveld et al.¹³⁹ where 100 CGG repeats placed in the *FMR1* gene leads to the formation of inclusions in neurons and eventually neurological disease. Similar work in the *Drosophila* with placement of 90 repeats into a reporter gene led to over expression in the eye and inclusion formation and neuro-degenerative changes in the eye followed.¹⁴⁰

Although FXTAS was originally considered to be rare, a recent penetrance study that assessed all known carriers older than 50 years in California (n = 192) found involvement primarily in males.⁵ Tremor and ataxia were seen in 17% of male carriers in their 50s, 38% of those in their 60s, 47% of those in their 70s, and 75% of those in their 80s. Although involvement in carrier females was not seen as significantly different from controls in this study⁵ and in a study of carriers in Chicago¹²⁸, FXTAS does occur in occasional female carriers.^{141–144} Perhaps the presence of a normal X chromosome or hormonal factors protect females from FXTAS, but when they occasionally do have neurological problems, severe cognitive deficits are not usually seen.¹⁴¹

It is possible that the POF present in female carriers may be caused by a toxic RNA effect in the ovary. A recent controlled study of endocrine function in 11 normally ovulating premutation carriers (ages 23 to 41 years) demonstrated a significantly shortened cycle, significant elevation of FSH throughout the cycle (91% with elevations >2 SD above the mean) in addition to elevation of inhibin B in the follicular phase and inhibin A and progesterone in the

luteal phase in carriers compared to controls.¹⁴⁵ These findings suggest a decreased number of follicles and granulosa cell dysfunction or decreased cell number in the corpus luteum compared to controls.¹⁴⁵ In addition 45% (5 of 11) had a history of infertility (defined by 1 year of unprotected intercourse without a pregnancy), compared to the rate of 10% in the general population, according to the Center for Disease Control. These findings suggest that women who present with infertility and elevations of FSH or POF should be screened for the premenutation. On occasion POF can occur in adolescence so pediatricians should be aware of the association of POF and the premenutation.

The emotional problems mentioned above in individuals with the premenutation may also be related to a toxic RNA effect. Since the inclusions occur at a high rate in the hippocampus,¹³² part of the limbic system, this is not surprising. The recent study by Hessler et al.¹³⁰ correlating elevations of *FMR1*-mRNA with obsessive-compulsive symptoms on the SCL-90 supports the hypothesis of a toxic RNA effect leading to emotional problems in males and in females with an unfavorable activation ratio. Although the full FXTAS phenotype in females is rare,¹⁴¹⁻¹⁴⁴ we have commonly encountered symptoms of muscle pain consistent with fibromyalgia, in addition to pain in the extremities that is likely part of the neuropathy symptoms including decreased sensation and vibration sense in the lower extremities. The variability and penetrance of the FXTAS phenotype in females has not been well delineated and requires further study.

The preliminary diagnostic criteria for FXTAS as reported by Jacquemont, Hagerman et al.¹²⁴ and modified by Hagerman and Hagerman¹³⁷ are outlined in Table 1. Those that have tremor, ataxia, and cognitive deficits represent the most severe end of the spectrum of RNA toxicity and the mildest version may include psychiatric problems, reproductive problems or neuropathy only.

The progression of mild FXTAS symptoms varies remarkably and we have seen mild symptoms stable for decades in some and in others a rapid progression presumably related to additive genetic effects.^{137,146} For instance, one gentleman with a PhD in electrical engineering had a rapid decline of his FXTAS condition to dementia in his late sixties over a 6 year period. At autopsy he demonstrated neuropathological findings of FXTAS including the typical eosinophilic and ubiquitin positive intranuclear inclusions in neurons and astrocytes and white matter disease in addition to the plaques and tangles of Alzheimer's disease.¹⁴⁷ Other genetic and environmental modifying effects both protective and detrimental to the phenotype of FXTAS have yet to be elucidated.

It is important for pediatricians to understand the RNA toxicity mechanism of involvement in FXTAS because young males with the premenutation who have social deficits and/or cognitive deficits may have a similar mechanism of involvement at an earlier stage in development. This is an area that warrants further research. Pediatricians should also ask about neurological problems similar to FXTAS in parents or grandparents when a child is diagnosed with

FXS and when taking a family history on a child with MR or autism of unknown etiology who could be affected with FXS.

TREATMENT OF FXS

The treatment of patients with FXS involves a multi-modality intervention that includes speech and language therapy, occupational therapy, behavioral interventions, special education support and psychopharmacological interventions.¹⁴⁸⁻¹⁵³ The ADHD problems typically respond well to stimulants as demonstrated by two controlled studies^{154,155} and surveys.^{148,156} The use of multiple medications is common because the behavior problems also include anxiety, which typically responds well to a SSRI, and mood instability or aggression, which often requires an anticonvulsant or an atypical antipsychotic, such as risperidone (Risperdal) or aripiprazole (Abilify). Aripiprazole is the newest atypical on the market and it does not cause the severe weight gain that is seen with most of the other atypicals. Parent reports have been particularly positive about aripiprazole (see the National Fragile X Foundation, www.fragilex.org, go to message board, and then medications) because it not only stabilizes mood but it seems to improve attention and academic progress. In our experience, high doses lead to agitation, so a low dose i.e. 2.5 to 5 mg at night seems to work best for children and many adults with FXS. Controlled trials are still needed in children and adults.

The advances in our understanding of the neurobiology of FXS, particularly the overactivity of the mGluR5 pathway, has led to studies of mGluR5 antagonists in animal models of fragile X including the knock out (KO) mouse model and the *Drosophila* model, the *dfxr* mutant fly.¹⁵⁷ One mGluR5 antagonist 2-Methyl-6-(Phenylethynyl)-Pyridine (MPEP) has dramatically improved audiogenic seizures and improved hyperactivity in the *FMR1*-KO mouse¹⁵⁸ bringing the phenotype consistent with the wild type mouse. MPEP has reversed abnormal courtship and neuronal growth phenotypes and improved cognition and memory in *dfxr* mutant flies.³³ Neither MPEP, nor any other mGluR5 negative modulators are currently approved for use in humans. Further research is warranted in humans to see if mGluR5 negative modulators could be beneficial as a specific treatment for FXS.

An alternative agent which is currently available for use in humans and acts to negatively regulate the mGluR5 pathway is lithium. mGluR5-dependent translational activation is signaled through a mechanism dependent on phospholipase C (PL-C) activation with production of diacylglycerol (DAG) and inositol triphosphate (IP3), and resultant calcium mobilization with activation of protein kinase C (PK-C). Lithium down-regulates this pathway by inhibiting inositol phosphate (IP) turnover,¹⁵⁹⁻¹⁶¹ and thus attenuating PL-C activity and PK-C activation.¹⁶² Lithium might attenuate overactive mGluR5-mediated translation due to absence of FMRP in FXS. In support of lithium's effect on this pathway is the recent demonstration that PK-C overactivity produces prefrontal working memory deficits in rats and monkeys that are reversed by lithium

treatment.¹⁶³ Working memory is a particular area of cognitive weakness in individuals with FXS^{164,165,117} and in many other neurodevelopmental disorders.

Lithium has been helpful for the fragile X animal models, specifically improving defects in naïve courtship behavior as well as two phases of memory, immediate recall (0 min) and short-term memory (60 min) in *dfxr* mutant flies.³³ Lithium has also been shown to improve seizures in the KO mouse [¹⁵⁷, Bauchwitz, personal communication]. Previous anecdotal reports found lithium to be helpful for aggression and mood stabilization in adolescents and adults with FXS¹⁶⁶ but lithium has not been studied regarding its effect on cognition or seizures in FXS. The new findings in the mouse and fly models of FXS and our new understanding of the neurobiology of FXS have suggested that benefits of lithium treatment should be re-evaluated in controlled trials across a broad range of cognitive and behavioral, neurobiological, and electrophysiological measures in children and adults with FXS. In FXTAS, there are a variety of medications that may be helpful for tremor and ataxia; but again no controlled studies have been carried out.¹⁴⁶ There is a great need for further research in this area.

Another important area of treatment is genetic counseling which is valuable for family planning. We recommend DNA testing of all siblings of a proband diagnosed with fragile X and of relatives at risk for involvement with the premutation or full mutation.¹⁶⁷ In our experience, siblings who appear to be normal compared to a proband with fragile X may sometimes demonstrate the full mutation and have learning problems that need treatment. In addition the premutation may be identified and a further workup for social deficits and attentional problems are indicated particularly in boys with the premutation who demonstrate academic and/or social difficulties.⁶⁻⁸ Families should also be informed of the risk of FXTAS, that occurs in approximately 38% of older male carriers, although this should not preclude testing.¹⁶⁸ The benefits of treating problems associated with the premutation in childhood or adulthood outweigh the concerns regarding FXTAS in the aging adult.¹⁶⁸ Most individuals with the

premutation will not experience FXTAS and for those that do the course is variable.

CONCLUSIONS

The fragile X field has moved rapidly in the last few years with new molecular mechanisms of involvement including RNA toxicity and broadened phenotypes that go well beyond mental retardation in childhood and inform geriatric conditions of cerebellar ataxia, Parkinsonism and dementia. Although fragile X is a single gene disorder, the absence of FMRP has a broad impact on the genome, so it is a model for complex genetic disorders. An exciting aspect of both FXS and FXTAS is the molecular overlap with other conditions that are becoming apparent as advances in neurobiology coincide with advances in genetics. These overlaps are apparent in the Prader-Willi phenotype of fragile X and may become more obvious with autism, such that advances in each condition will be informative for the other. We are just beginning to understand the RNA toxicity in FXTAS and the white matter disease that occurs in some aging carriers and may be deleterious even in early development for a rare patient. A better understanding of modifying genes and protective factors will further our understanding of the variable phenotypes we see clinically in both FXS and FXTAS. Most importantly, the molecular advances are leading to specific pharmacological interventions for fragile X which reinforces the need for early identification and early intervention.^{157,169} Newborn screening for fragile X is in the planning stages and will facilitate early treatment and genetic counseling for immediate and extended family members.¹⁷⁰ There is much work to be done and it begins by educating ourselves about this fascinating and relatively common condition.

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