Published in final edited form as:

Adv Pediatr. 2009 ; 56: 165–186. doi:10.1016/j.yapd.2009.08.008.

Fragile X: A Family of Disorders

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Introduction

For those of you who have seen a child with fragile X syndrome (FXS), you probably know that it is the most common inherited cause of intellectual disabilities (ID) and also the most common single gene cause of autism [1]. However, what you may not know is that there is a broad spectrum of involvement throughout the generations in a single family that includes both medical and psychiatric involvement in those with the premutation and in those with the full mutation. In the carrier mother, who usually has a normal IQ, there is an increased risk for primary ovarian insufficiency [2], depression and anxiety [3,4], and late onset neurological problems including hypothyroidism, fibromyalgia, neuropathy and the fragile X-associated tremor ataxia syndrome (FXTAS) [5]. In the grandparent who is the carrier, particularly the grandfather, there is a high risk for FXTAS and also dementia [6]. There is often involvement in extended family members at all levels in the generations so a detailed family history is important that reviews many of the disorders that are associated with the premutation (55 to 200 CGG repeats on the front end of the *FMRI* gene) and the full mutation (>200 repeats).

You may have ordered a fragile X DNA test on a child with autism and found the premutation and thought that this was not a cause of his problems, since it was not a full mutation. We now know that some individuals with the premutation can have developmental problems [7] and this is related to an RNA gain-of-function secondary to elevated mRNA in those with the premutation. Chen et al have shown that premutation neuronal cell cultures demonstrate changes in branching and synaptic size compared to controls [8]. Although those with the premutation usually have a normal IQ and are successful in life, developmental problems including ADHD, executive function deficits, memory problems and autism spectrum disorders (ASD) can be seen, particularly in boys [7,9]. In individuals who have ID with the premutation, there is often a deficit of fragile X mental retardation protein (FMRP), the protein produced by the fragile X gene and this is most commonly seen in the upper end of the premutation range in both humans and in the premutation mouse model (120–200 CGG repeats) [10,11]. This review focuses on the latest research in both FXS and in premutation involvement and their treatment.

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Epidemiology

The premutation is common in the general population with a prevalence of 1 in 130–260 females and 1 in 250–810 males [12]. There is variability in the prevalence figures depending on where the study was done and the ethnic or racial background of the patients. The study recently reported by Cronister et al 2008 [13], has shown that the premutation is less common in those of Chinese background and more common in the middle east, particularly Israel as reported by others [14]. The allele frequency of the full mutation is approximately 1 in 2,500 [12] although the affected prevalence has been shown to be 1 in 3,600 [15] because some individuals with the full mutation do not have significant ID, particularly females.

In order to test for the premutation or the full mutation you order a fragile X DNA test or also called the *FMR1* DNA test. This is done in almost all university molecular laboratories and in commercial laboratories with a cost ranging from \$200 to 500. It is usually covered by insurance companies or Medicaid. The DNA test includes a PCR and a Southern Blot so that the number of CGG repeats are reported and the presence of abnormal methylation, which typically occurs only in the full mutation. With complete methylation there is little or no *FMR1*-mRNA in the full mutation leading to a deficit of FMRP (Figure 1). In the premutation there is no methylation and the level of *FMR1* mRNA is increased. The premutation mRNA also contains the expanded CGG repeats although they are not translated. In the high end of the premutation this can lead to a mild block in translation so that a mild deficiency of FMRP can occur. If the FMRP level is significantly decreased the child with the premutation can have many features of FXS, such as prominent ears, hand flapping, connective tissue problems or poor eye contact. This can be confusing to the clinician because most individuals with FXS have the full mutation, although, on occasion FXS can be due to the premutation with a significant FMRP deficit.

Phenotype of FXS

Most physicians typically may not suspect fragile X syndrome unless typical dysmorphic features or a family history of ID is seen. However, approximately 30% of families with FXS do not have a family history of ID and about 30% of children with FXS do not have obvious physical features of this disorder. On examination there is a broad spectrum of morphological, cognitive, behavioral and psychological features, even though FXS is a single gene disorder. This variability is related to the differences of individual genetic background, environmental influences in addition to variations in the *FMR1* gene including the degree of mosaicism (both size and methylation mosaicism), *FMR1* mRNA levels, FMRP levels, and activation ratio (fraction of normal *FMR1* alleles that are active in females). An example of an environmental influence is the surgical pinning procedure that some families carry out to treat the prominent ears that are seen in the majority of children with FXS. The most significant molecular variable is the level of FMRP. In those with the full mutation or mosaicism, FMRP levels correlate with the IQ and a number of physical features typical of fragile X including ear prominence. FMRP correlates with the level of involvement of premutation carriers as previously mentioned [1,7,16]. The following details phenotypic findings in those with the full mutation.

Physical Features

Most young individuals with FXS have changes in their connective tissue including prominent ears, hyperextensible finger joints, soft skin and flat feet [17] which relate to changes in the elastin fibers of connective tissue [18]. Pectus excavatum, mitral valve prolapse, strabismus, long face, prominent chin and enlargement of testicular size have also been documented in individuals with FXS [17]. Macroorchidism usually begins to present

after age 8 and the maximal size of the testicles (about 2 or 3 times normal) is seen in mid adolescence [19].

Cognitive Features

Most children with FXS will not be diagnosed until they are almost 3 years of age. Often the first diagnosis of children with FXS is autism because the behavioral features of poor eye contact, hand flapping and social deficits are the most prominent features [17,20]. Since all individuals with autism or autism spectrum disorder (ASD) are routinely screened for FXS, the etiology for their autism will be readily apparent with *FMR1* DNA testing. Approximately 30% of boys with FXS will also have autism and an additional 20 to 30% will have PDDNOS as described below [21]. Recently lower fine motor scores have been reported in 1 to 6 year-old children with FXS, particularly with autism and FXS together [22]. In addition cognitive, language and adaptive skills are lower in those with FXS plus autism compared to those with FXS alone [23–25].

Parents typically notice that their children are not talking by the time they are 13 to 18 months [26]. They, therefore are brought to see primary physicians or pediatricians and are diagnosed with a developmental delay at an average age of 21 months [26]. Unusual sensory-motor development, such as object play skills (e.g., spinning) and unusual motor patterns (e.g., repetitive movements of the legs, unusual posture) during 9–12 months of age may be helpful to recognize FXS in early infancy. The lag time between the initial parental concerns and the diagnosis of developmental delay can be addressed through standardized developmental screening tests at the 9-, 18-, and 24 or 30-month well child visits to detect developmental disorders early [27]. The developmental trajectory in fragile X is slow (about 50% of normal in boys) and IQ decline may be seen in late childhood and adolescence, although mental age continues to increase even though the rate is slower than normal [28].

Approximately 85% of males and 25–30% of females with the full mutation have an intelligence quotient (IQ) less than 70 (intellectual disability: ID) [1,16]. The severity of intellectual disabilities is related to FMRP deficiency [16,29]. Some individuals with only a mild decrease of FMRP level may present with a normal or borderline IQ with or without learning disabilities (LD). LD with a normal or borderline IQ is a typical presentation in females with FXS. Math skill is the main area of academic underachievement in the girls and high functioning boys with FXS [1,30]. Other cognitive impairments involve many domains including executive function, visual memory, visuospatial-processing abilities, visual-motor coordination, auditory short-term memory, processing of sequential information, sustained attention, and working memory [30–33]. Fortunately, children seem to have relative strengths in verbal-based skills, general knowledge and activity of daily living skills [30,31]. Academic acquisition is not predicted by FMRP level or nonverbal IQ, but the level of maternal education and presence of autistic behaviors do predict academic strengths in children with FXS [30].

FMRP regulates a number of proteins important for synaptic plasticity [34]. FMRP typically inhibits the translation of proteins at the synapse, so in the absence of FMRP there is up regulation of a number of proteins including proteins in the metabotropic glutamate receptor 5 pathway (mGluR5) leading to long term depression (LTD) or weakening of synaptic connections [35]. Recently, cognitive deficits in memory have been found in the drosophila model of FXS related to up regulation of *staufen* and *argonaute1* proteins [36]. These cognitive problems were reversed by treatment with puromycin which blocks protein production. Antagonists of the mGluR5 system have also reversed the cognitive deficits in the animal models of FXS including the knock out mouse and drosophila [37,38].

Epilepsy occurs in approximately 10–40% of individuals with FXS [39,40]. The seizures are usually generalized or partial complex seizures and they may occur at night [40,41]. Seizures are about three times as common in those with FXS plus autism compared to FXS without autism [41]. Seizures may be related to dysfunction in both the mGluR5 pathway and in the Gamma-aminobutyric acid A (GABA_A) pathway. Dysfunction in the GABA system has been found in the KO mouse model of FXS and there appears to be down regulation of the GABA pathways particularly the GABA_A pathway [42]. The group of Steve Warren has found that supplementation with medication that enhances the GABA system will rescue the drosophila model with FXS from death [43]. In addition use of mGluR5 antagonists will reverse the seizure phenotype in the KO mouse model of fragile X [44].

Behavior and Psychological Features

Behavior and psychological characteristics of FXS include shyness, social avoidance, anxiety, autistic behaviors (poor eye contact, perseverative behaviors or speech, sensory hypersensitivity, stereotypic or repetitive motor behaviors such as hand-flapping and hand-biting), distractibility, hyperactivity, inattention, impulsivity, tactile defensiveness, self-injurious behavior, aggression, mood instability, irritability and inflexibility [17,24,45,46]. Females typically have milder behavioral and cognitive problems than those observed in males since they have some FMRP production from their normal X chromosome. Nonetheless, they are likely to develop emotional problems and maladaptive behaviors, including depression, social anxiety, and withdrawal [31]. Although most individuals with FXS are shy at first they will warm up and become friendly over time, however those with FXS plus autism will typically not warm up and will remain relatively aloof over time [47].

The reason why children with FXS are more likely to have anxiety may be due to their enhanced sympathetic response to stimuli and their inability to accommodate or habituate over time to stimuli. This autonomic dysregulation is likely related to the mGluR5 upregulation and the GABA downregulation previously described. Individuals with FXS also have enhanced cortisol release after stressful stimuli [48]. This hyperarousal to stimuli can lead to tantrum behavior and even aggression which often requires medical treatment as described below [49]. Interestingly, dysregulation of the autonomic nervous system is more common in those with FXS plus autism [50].

In a family study there was a strong interaction between the child's behavior with FXS and the adaptation of the mother [51]. The degree of the child's behavior problems had a strong effect on the mothers' stress level in addition to her depressive symptoms, anxiety, anger, and quality of life [51]. Because the mother will have the fragile X mutation too, typically the premutation, which puts her at risk for anxiety and depression [3], it is important to recognize the problems that she is experiencing and recommend treatment for the mother if needed.

Once a proband is diagnosed the evaluation of the whole family tree should take place with a genetic counselor. There will be many other individuals who will have additional involvement from the premutation (described below) and the full mutation. A spectrum of psychiatric, endocrine, medical and neurological problems will be found related to these mutations [5,6,52,53]. We routinely recommend testing of family members who are at risk for either the premutation or full mutation involvement because of the many treatment options that may reduce the burden of disease over time [49,54]. For instance, identification of a carrier will help surveillance for hypertension and hypothyroidism which are increased in carriers [5] and early treatment of these conditions will decrease the medical complications of these problems. Early treatment of depression may influence the cognitive

decline which occurs in older carriers related to FXTAS [54]. Early identification of those affected with FXS will lead to early conventional treatments and targeted treatments described below.

Relationship between FXS and autism

There is a very close relationship between fragile X syndrome and autism. Two to seven percent of children with autism will have a mutation in *FMRI* [1]. The prevalence of autism in the individuals with fragile X syndrome has been reported to range from 15–35%, although when the Autism Diagnostic Observation Scale (ADOS) is used it is approximately 30% [21,24,25] with an additional 20 to 30% with PDDNOS [21]. Even those without ASD will have a number of autistic-like features, such as poor eye contact, hand-flapping, perseverative and repetitive behaviors, language and self talk [17,24,25].

There is an increased prevalence of autism in individuals with FXS likely because FMRP regulates the translation of a number of proteins known to be associated with autism, such as neuroligins, neuorexins, SHANK protein, PTEN, CYFIP, PSD95 and many more [1,20,34,55]. The lack of FMRP interferes with synaptic plasticity, leads to dysconnectivity in the CNS, and causes dysregulation of GABA and glutamate systems all of which are associated with other forms of autism [20]. Why autism occurs in some individuals with FXS and not all is not known. There are predictive factors including severity of intellectual disabilities [24,25,56], degree of FMRP deficiency [57], increased age [57], presence of seizures or additional genetic disorders [41], poor adaptive social skills [24,56] and more behavioral problems [56], that are associated with increased autism or ASD in FXS. However, FMRP level is not related to the presence or severity of autism when the IQ was controlled suggesting that factors in addition to fragile X lead to the presence of autism [58].

Cognition, adaptive behavior, language, and social interaction are more severely impaired in those with both FXS and autism compared with FXS alone as described above [24,25,47,56,57]. Language deficits in fragile X individuals with ASD are likely to involve more severe receptive deficits, including verbal reasoning, recognition of emotions and labeling of emotions [23,45] than those with FXS alone. Social affective impairment in the child with FXS with ASD makes it challenging for them to learning language effectively. The language learning processes require more investigation to clearly understand why language outcomes are quite variable in FXS with or without autism. Restricted, repetitive and stereotyped behaviors are not the important clue for the autism diagnosis in individuals with intellectual disabilities including FXS [24]. To diagnose autism in children with FXS, an assessment of communication deficits, social withdrawal behaviors, impairment in complex social interactions and adaptive social skills is needed because these pivotal deficits are highly associated with autism [24,45,56]. High levels of social withdrawal characterized as avoidance in multiple social settings and low interest in others can be seen in individuals with autism and FXS [47]. Autistic behaviors in FXS may change and vary day to day or even related to the time of day or emotional reactivity to events. Therefore a comprehensive evaluation involving different aspects of the assessment with different clinicians may be necessary to reach consensus on the diagnosis of autism. Such a diagnosis is essential to help children receive appropriate intensive interventions needed for autism, although such interventions are also beneficial in those without autism who have FXS [21].

There are a number of neuroanatomical and neurobiological similarities between FXS and idiopathic autism; particularly their common phenotypes including macrocephaly which represents rapidly increase brain growth during early childhood period [59]. A *PTEN* mutation, has been reported approximately 18% of the idiopathic autism with extreme macrocephaly [60]. In FXS, PTEN activity is down regulated (S. Zupan, personal

communication, FRAXA conference, Maine 2008) [34]. This is likely why children with FXS especially those with FXS plus autism have a larger head [59].

A number of genetic conditions, such as Down syndrome [29], Prader-Willi Phenotype (PWP) of FXS [61] and medical conditions affecting the CNS, such as brain trauma, cerebral palsy, recurrent seizures when added to FXS will increase the risk for autism [41]. On the other hand, individuals with FXS and ASD may be more vulnerable to have more medical problems affecting the CNS when compare to the individual with FXS alone (38.6% VS 18.2%) [41]. For instance, seizures occur in about 28% of children with FXS and ASD, but occur in only 12% in those with FXS without ASD [41]. Neuronal excitability and susceptibility to develop seizures from underlying CNS conditions may be associated with emerging autism in FXS. On the other hand, abnormal electrical discharges from epilepsy possibly disrupt brain connectivity and lead to autism when you have FXS [20,41]. Nevertheless, it is important to search for seizures or abnormal spike wave discharges in those with FXS and autism because anticonvulsant medication, such as valproate may be helpful for the seizures and for the autism. [41,49].

ASD occurs in approximately 70% of those FXS with the Prader-Willi-Phenotype (PWP), suggesting a role for the second genetic hit in leading to autism [29,61]. The PWP in FXS does not have a 15q deletion nor uniparental disomy like Prader-Willi syndrome. In the Prader-Willi phenotype of FXS, individuals have severe hyperphagia emerging in the first 3 to 8 years of life, lack of satiation after a meal, obesity, and hypogenitalia or delayed puberty. Recently, it was reported that the expression level of cytoplasmic *FMRI* interacting protein (CYFIP1), normally coded within the Prader-Willi syndrome region (15q region between break point 1 and 2), was lower in individuals with PWP and FXS than those with FXS but without PWP and typically developing individuals [61]. CYFIP interacts with Rac1 which has GTPase activity and is also essential in synaptic function and neuronal migration [61]. Lowered CYFIP expression may have an additional impact on synaptic plasticity and may predispose individuals with PWP and FXS to develop more autism. Recently Buxbaum [62] reported that deletion of CYFIP in mice causes autism. Why CYFIP is down regulated in a small subgroup of patients with FXS who have the PWP is not known. FMRP regulates CYFIP translation and the absence of FMRP typically leads to elevation of CYFIP levels in FXS without the PWP compared to controls without FXS [61].

There is remarkable overlap between neurobiological mechanisms leading to several known forms of autism and FXS with autism. In summary, the *FMRI* gene can regulate the expression of other genes and affect synaptic formation and plasticity [34,63]. Therefore, if FMRP is decreased or absent, there is significant dysregulation of various pathways/proteins that disrupt brain development pervasively leading to developmental delays, particularly autism. Disruption in critical networks occur in the absence of FMRP including upregulation of the mGluR5 pathway [35], upregulation of the mTOR pathway [34], down regulation of the PTEN pathway, down regulation of the GABA_A receptors [42], down regulation of the dopamine pathway [64], dysregulation of neuroligin 3, 4 and SHANK3 [34]. We are able to assess dysregulation in GABA and mGluR5 systems with electrophysiological methodology including prepulse inhibition (PPI). In individuals with FXS and/or ASD, who have problems of frontal gating or sensory information processing, PPI is typically decreased [65]. This measure can also be utilized in the quantitation of brain improvements with the use of targeted treatments, such as mGluR5 antagonists in FXS explained below in treatment.

Neurobiological Advances

FMR1 is involved in the control of protein synthesis, and recent studies have found that FMRP shuttles from nucleus to cytoplasm as an mRNP particle [34]. FMRP has been shown to be an mRNA-binding protein, which regulates synaptic and cytoskeleton-associated proteins. Formerly thought to be just a general repressor of translation, FMRP is now considered to be restricted to specific messages. Most importantly the mGluR-signaling pathway is regulated by FMRP [35]. Besides the mGluR mechanism, other pathways have been identified for FMRP interaction (Figure 2).

Dendritic spines are small membranous extensions on a neuronal dendrite in the brain. The spines serve as synaptic storage sites, support the electric signal transmission and increase the number of possible contacts between neurons. On their surface, the dendritic spines express glutamate receptors (GluR), e.g. the AMPA receptor and the NMDA receptor. A broad variety of proteins (e.g. kinases) mediate the signaling from the GluRs. Cognitive function, motivation, learning and memory are based on spine plasticity. Especially the long-term memory formation is mediated in part by the growth of new or existing dendritic spines to reinforce a particular neural pathway. Enhancing the ability of the presynaptic cell to activate the postsynaptic cell strengthens the connection between two neurons. After the formation of numerous dendritic spines during fetal cortical neurogenesis, the dendritic spines need to mature or they are pruned. Immature spines can be identified through their malformations (e.g. long “necks” or lack of “heads”). Those immature spines show a significant impairment in signal transduction. Several studies show abnormalities in the spine formation in FXS, which can be directly correlated to the cognitive impairment. A neurophysiological study by Wilson & Cox (2007) demonstrated a clear attenuation in the cortical LTP (Long-Term Potentiation) in slices from FMRP-KO mice compared to the wildtype [66]. The authors suggest the decrease in LTP is caused by a diminished mGluR5-mediated activity in the neocortex. This leads to an enhanced LTD (Long-Term Depression) through the absence of FMRP inhibition of protein translation, implicating a complex interaction between FMRP and mGluRs. The changes in synaptic plasticity occur differently in various brain areas. LTD is associated with a reduction in the number of postsynaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type glutamate receptors that mediate excitatory activity in brain and shuttle between post-synaptic membrane and beneath in cytoplasm regulating synaptic excitability [63]

FXS is associated with protein-synthesis-dependent lengthening/“thinning” of dendritic spines. The synapses on thin dendritic spines have smaller postsynaptic density, fewer AMPA receptors, and a reduced number of synaptic vesicles docking at the presynaptic active zone [67]. The lengthening of dendritic spines suggests an interaction of FMRP with the Rac1 pathway and this is through CYFIP1 [61]. Rac1 has been shown to regulate actin dynamics, and dendritic spines are actin-rich structures.

A study by Nakamoto et al. 2007 on cultured hippocampal neurons demonstrated a hypersensitive AMPAR internalization with FMRP deficiency correlated to the excess mGluR signaling, pointing to the cellular defect in FXS that causes learning and memory deficits [68]. The authors utilized mGluR antagonists to reverse this pattern and “rescue” the excessive signaling in FXS cells. This study was based on the mGluR hypothesis of FXS, postulated by Bear et al. 2004 [35]. In short, Bear et al. postulate that the loss of FMRP in FXS leads to an excessive expression of mRNA near synapses, making it impossible to regulate protein synthesis adequately, thus increasing LTD due to receptor loss (Figure 3).

Glutamate is the most common excitatory neurotransmitter in the mammalian brain. A major function is the stimulation of local protein synthesis at synapses required for synaptic

function and inducing of LTD [38]. In the FMR1 knockout mouse, the mGluR-dependent activity is increased, leading to increased LTD (mGluR-LTD) [35]. In Bear's mGluR theory, the heterogeneous FXS phenotypes are due to mGluR misregulation [35]. LTP and LTD are synaptically triggered and lead to long-lasting changes in synaptic strength. Cognitive functioning is dependent on synaptic plasticity, and impairment leads to learning and memory deficits. In neonates, LTP leads to retaining nascent synapses, and LTD-activity-guided synapse elimination, providing the base for postnatal learning and memory storage. LTD is triggered by mGluR activation, which requires rapid translation of mRNA in postsynaptic dendrites. The lack of appropriate FMRP levels lead to an exaggerated mGluR5 activation [35].

FMRP is widely expressed in the brain, and mGluR-dependent protein synthesis is exaggerated in absence of FMRP in various brain areas particularly the hippocampus [35]. If LTD is associated with changes in synaptic receptors for glutamate, then FXS is treatable with drugs interacting with GluR receptors. One example for a GluR-interacting substance is MPEP (2-methyl-6-(phenylethynyl)-pyridine) – a potent and specific, non-competitive antagonist of mGluR receptors. As postulated, several animal studies showed a beneficial effect of MPEP. The substance rescued behavioral abnormalities in the *FMR1* KO mouse [44], the courtship and mushroom body defects in the drosophila *dfmr1* mutants [69].

During human development, the synaptic connections in the brain undergo drastic changes – from thin, long dendritic spines with small synapses to short, mushroom-shaped dendritic spines with bigger synapses [70]. In FXS, the dendritic spines in the brain appear immature, and could lead to the conclusion that only little, or no pruning happened due to the neurochemical imbalance of FMRP and mGluR mechanisms. Pruning of synaptic connections is essential for the fine-tuning of neural networks and does happen but to a reduced degree in FXS with an absence of FMRP. The larger number of immature dendritic spines and weak synaptic connections could lead to cortical hyperexcitability.

In summary, the neurobiological base of FXS is a disruption of interrelated neuronal signaling pathways involved in learning and memory.

Neuroimaging in FXS

There have been numerous neuroimaging findings in FXS depending on the population studied and the techniques utilized. Individuals with FXS have reduced activation in the right ventrolateral prefrontal cortex, which is an area of the brain important for executive function, compared to both autistic and typically developing subjects. Hoefft et al. hypothesized that the lateral prefrontal cortex abnormality together with larger caudate volumes (which is associated with lowered FMRP level), may play a role in hyperactivity, frontostriatal dysfunction and the response inhibition deficit seen in FXS (part of executive function involved in sustained attention, target detection and rule maintenance) [71]. Normally, the striatum has a main function in motivation and cognitive abilities; therefore individuals with FXS, who typically have frontostriatal dysfunction, may exhibit hyperactivity, stereotypic and repetitive motor behaviors. Girls with FXS have reduced basal forebrain and hippocampal activation related to FMRP deficits and these problems, are important for attention and memory encoding. Enlarged hippocampal volumes bilaterally have been reported in children with FXS. This brain area is vital to memory processing, emotional and stress regulation [71,72].

Autism and fragile X syndrome have some similar neuroimaging findings. For instance both disorders have a large cerebrum but the posterior cerebellar vermis, an area involved in sensory perception, cognition and motor function; is smaller or abnormal when compared to controls [20,72]. This reduced volume of the posterior cerebellar vermis accompanied by a

large caudate nucleus may be associated with decreased FMRP, severe autistic behaviors, profound cognitive deficits and aberrant behaviors [72]. Both FXS and autism individuals have similar decreased activation patterns in the fusiform gyrus which is correlated with deficits in gaze fixation to human faces [73]. However, the FXS group had greater activation than the autism and control groups in brain regions including the left hippocampus, the right insula, the left postcentral gyrus, and the left superior temporal gyrus, involved in fear, processing of emotional faces and complex auditory stimuli [73]. Amygdala dysfunction, a potential area underlying social deficits, has been described in fMRI amygdala activation studies to social cognition tasks in both ASD and adult males with the premutation [74].

A lack of normal recruitment of the neural network, involved in visual memory tasks to solve more difficult math problems or perform executive function tasks has been demonstrated on fMRI in FXS [75]. Furthermore, parietal lobe dysfunction is also associated with dorsal stream processing deficits that were investigated through biological motion processing tasks and may be involved in visual motor deficits in both FXS and individuals with autism [1,32].

Other brain abnormalities in FXS include a larger hypothalamus. This structure is likely involved in the abnormal stress response with enhanced cortisol release, sleep disturbances and abnormal melatonin release seen in FXS [71,76]. There is also a smaller insula and medial prefrontal cortex (involved in aberrant activation during gaze processing, hyperarousal, and cognitive and social features in FXS) and a smaller right superior temporal gyrus, involved in cognitive deficits in FXS [20,71]. The complexity of the morphometric changes in the brain related to the deficiency or absence of FMRP is profound and related to the great number of pathways that are dysregulated with the absence of this protein. [71].

Neurophysiological studies in FXS

Only few EEG studies in FXS have been published so far. Among common findings are seizures, abnormally large somatosensory evoked potentials, and the occurrence of interictal paroxysmal EEG activity in prepubertal subjects with FXS. There are similarities between EEG findings in FXS and benign childhood epilepsy with centrotemporal spikes [40]. Additional abnormal EEG findings in FXS have been described [39], including abnormalities in theta-rhythm, diffuse spike-wave and focal spike-wave discharges particularly in the temporal and central regions. A comparison of findings in single photon emission computed tomography (SPECT) and EEG show a distinct overlap in the results – with a cerebral dysfunction in FXS, with slow-wave paroxysms and cerebral perfusion abnormality with focal deficits seen in frontal regions in females with FXS [77].

Another approach to evaluate perception and cognitive processes in the brain are event-related potentials (ERPs). The brain activity at a fixed time-frame, dependent on the occurrence of a sensory or a cognitive stimulus (e.g. the presentation of a tone or a picture) is measured with an EEG and averaged. The specific pattern of the amplitudes and latencies of the ERPs provides an insight in the underlying information processing in the brain. For example, an auditory ERP assesses the stimulus processing in auditory afferent pathways and corresponding cortical areas. The N1/N100 is the first prominent negativity in the auditory evoked potentials, generated by at least three sources in the temporal and frontal lobes [78].

In children with FXS, an augmentation of the auditory N1 has been shown [79], and abnormal P300 in auditory evoked potentials in adults with FXS [80]. During development, a maturation of auditory stimulus processing occurs in FXS, with an increased responsiveness to auditory stimuli [79]. Compared to a healthy control group, the FXS group

showed a more frontal N1 scalp distribution, implicating an immature processing. The increased global field power shows a reduced inhibition and increased excitability. The FXS group did not show a N1 habituation, which is considered to be the first non-specific arousal as part of the orienting reaction and with the habituation an indicator of the refractory properties of neurons in auditory cortex.

A MEG study on auditory evoked magnetic fields showed a higher amplitude for the N100m auditory evoked field component, and less lateralized N100m anterior-posterior dipole locations [81]. The authors conclude that there is a more wide-spread activation of neurons that are activated by acoustic stimuli, consistent with the increased stimulus intensity experience in FXS. FMRP is considered to be playing a role in cortical hyperexcitability and abnormal synaptic transmission; the neuropathological bases for the hyperexcitability are the enhanced dendritic connections and immature pruning in FXS.

Another research approach to show sensorimotor processing deficits in FXS is the prepulse inhibition of acoustic startle reflex (PPI), a behavioral model of basic sensorimotor processing. The sensorimotor gating abnormalities in FXS, a heightened sensitivity to sensory stimulation and sensory defensiveness are caused by the abnormalities in maturation of synaptic connections in sensory circuits [82]. Compared to other mental disorders, the deficit in PPI in FXS is even greater than in schizophrenia [83]. PPI has been established as a reliable measure in FXS that will be useful in medication trials [65].

Studies have shown a hyperarousal and hyperreactive responses to sensory stimulation [50,84]. The electrodermal responses show an increased sensory sensitivity in FXS, and the magnitude of responses correlate negatively with FMRP expression [84].

The neurophysiological approaches to cognition, arousal and inhibition provide a useful base for outcome measures in future medication trials of FXS. Sensory processing and an improvement in cognitive functioning cannot be sufficiently assessed with broader neuropsychological tests, and especially the EEG and ERP paradigms will provide a better method to look at even subtle changes in the improvement of information processing, indicating a change in the underlying deficit in dendritic spine morphology in FXS.

Premutation involvement

Involvement in those with a premutation (55 to 200 CGG repeats) has a different molecular pathogenesis than those with the full mutation. In the premutation the elevated levels of FMR1-mRNA lead to toxicity in the cell, particularly the neuron [85]. The elevated mRNA causes dysregulation of several proteins including lamin A/C, myelin basic protein (MBP), and heat shock proteins leading early cell death [86]. Although most individuals with the premutation do not suffer from significant medical, psychiatric or cognitive problems related to this toxicity, others do and the findings of primary ovarian insufficiency (POI) and the fragile X-associated tremor ataxia syndrome (FXTAS) are thought to be related to this RNA toxicity (see figure 1) [2,85].

Approximately 40% of older males with the premutation will eventually develop FXTAS and the features of this disorder include an intention tremor, ataxia, parkinsonism, neuropathy, cognitive deficits, particularly executive function deficits with eventual cognitive decline to dementia in some and autonomic dysfunction including hypertension, impotence and eventual bladder and bowel incontinence [5,52,87,88]. Although some patients with FXTAS have a rapid decline over 5 or 6 years, others are stable for a decade or two. More rapid decline typically occurs when the features of FXTAS are combined with another disorder, such as multiple sclerosis [89], Alzheimer's [90] or Parkinson's Disease

[91]. In female carriers, FXTAS occurs in approximately 8% [5] but it is usually not associated with cognitive decline, although cases of dementia have been reported.

The neuroanatomical hallmark of FXTAS is intranuclear eosinophilic inclusions in neurons and astrocytes throughout the brain but with highest numbers in the hippocampus and limbic system [91]. These inclusions can also occur outside the CNS and they have been found in Leydig and myotubular cells of the testicles [92] and in peripheral nerve ganglia throughout the body [93]. These inclusions contain the excess mRNA and also a number of proteins including lamin A/C and MBP that are dysregulated by the elevated mRNA [86]. Although the inclusions themselves are probably not pathognomonic they are a marker for the RNA toxicity that is occurring in these cells.

It is important to recognize that there are other medical and psychiatric problems that can occur in some carriers and are not necessarily part of POI and FXTAS but may also be related to the mRNA toxicity. Neuropathy is relatively common in older carriers and can occur without other symptoms of FXTAS [5,87,94]. Hypertension is seen in the majority of older carriers and may be secondary to the autonomic dysfunction related to RNA toxicity [5]. In a study of carrier females both with and without FXTAS, hypothyroidism was seen in 50% and fibromyalgia was seen in over 40% of those with FXTAS [5]. This suggests that autoimmune problems are more common in female carriers. The elevated mRNA in carriers up regulates alpha B crystallin and heat shock proteins and perhaps this stimulates autoimmune activity. Multiple sclerosis (MS) occurs in 2 to 3% of female carriers and this may be related to the alpha B crystallin up regulation, since this is an important antigen in MS [5,89]. FXTAS has been reported in one woman with the premutation who had a long history of MS so both conditions can occur together [89].

Psychopathology that is more common in those with the premutation include anxiety, depression and obsessive compulsive behavior [3,4]. Although not all individuals with the premutation have significant psychopathology, these problems are clinically significant for 25 to 40% of carriers. There is now emerging evidence that premutation involvement has a neurodevelopmental component in some children, especially boys, causing a higher incidence of ADHD, shyness and social deficits including autism spectrum disorder [7,9,46]. Cell cultures of premutation neurons have also demonstrated abnormalities in dendritic branching and synaptic size compared to controls [8]. Further study of unselected premutation babies identified in the newborn period through screening will clarify what percentage of carriers will have neurodevelopmental problems and the benefit of early intervention. The new blood spot screening for the premutation developed by Tassone et al [95] will facilitate newborn screening studies that are now taking place at 4 centers in the US.

Targeted Treatments

There are a number of currently available medications that are helpful in the treatment of a variety of symptoms in children with FXS. Stimulants are usually beneficial for the 70 to 90% of boys and for the 30 to 40% of girls with FXS who have ADHD [49,96]. Research by Wang et al [64] regarding dopamine dysregulation in the absence of FMRP in brain tissue demonstrates normalization of dopamine function with the use of a stimulant, so, in a sense, stimulants are a targeted treatment for FXS. Clonidine or guanfacine can also be utilized for treatment of ADHD and they have an overall calming effect for the hyperarousal [49]. In Italy where stimulants are not utilized, treatment with L-acetylcarnitine has been used to treatment ADHD in children with FXS with some efficacy [97]. Selective serotonin reuptake inhibitors (SSRIs) have been successful in the treatment of anxiety in both children and adults with FXS [49]. Perhaps the most successful conventional medication in treatment of

FXS is aripiprazole (Abilify), an atypical antipsychotic which helps to stabilize mood, decrease anxiety and improve attention [49]. However, many children with FXS can become more agitated or hyperaroused at high doses so beginning with a low dose is essential, e.g. 1 mg at bedtime in mid childhood and ½ mg for children under 5 years [49]. For adolescents and adults a dose of just 5 mg at bedtime is usually adequate for a clinical effect.

The use of targeted treatments is an exciting area of research and it holds the promise of reversing the epilepsy, behavioral problems and cognitive deficits in FXS because the animal models have demonstrated these changes with the use of mGluR5 antagonists (Figure 4) as previously described. The initial clinical trial of fenobam, the first mGluR5 antagonist utilized in patients with FXS, was a single dose study in 12 adults that demonstrated no significant adverse effects and there were favorable behavioral changes in the majority of patients including calming of the hyperactivity and anxiety [98]. In addition, significant improvement of the PPI deficit was seen in 50% of the patients with FXS treated with fenobam. Within the next year there will be several trials of mGluR5 antagonists carried out in multiple centers in the US that are part of the Fragile X Clinical and Research Consortium set up by the National Fragile X Foundation (www.fragileX.org).

Another targeted treatment that may be helpful for children and adults with FXS is minocycline. In a study of newborn KO fragile X mice, a one month treatment of minocycline normalized synaptic connections by lowering matrix metalloproteinase 9 (MMP9) that is up-regulated without FMRP [99]. This exciting development has led to clinical instances of minocycline treatment of individuals with FXS and about 2/3rds of families have noticed subtle but positive improvements in language, attention and/or behavior. An open trial has been initiated in Canada recently and future controlled trials are needed. Minocycline can cause graying of the teeth in children who are younger than 7yo if their permanent teeth are not in place. Minocycline can also cause graying of other tissue with long term use and it can also cause enhanced sun sensitivity in most individuals and pseudotumor cerebri in rare instances. Further study of the neurobiology of FXS will likely lead to other targeted treatments and the combination of these treatments with educational endeavors to rehabilitate the cognitive deficits hold the promise for reversing the ID and behavioral problems in both children and adults with FXS.

Summary

There is a broad spectrum of clinical involvement throughout the generations in families affected by the fragile X mutations, both the full mutation and the premutation. A careful family history, assessment and genetic counseling should lead to better treatments in all individuals affected by the many manifestations of these mutations. Individuals with ID, autism, ASD, neurological problems of tremor, ataxia, neuropathy and cognitive decline in addition to those with early menopause, infertility and primary ovarian insufficiency should be tested for the fragile X mutation. New targeted treatments give hope of reversing the ID and behavioral problems in children and adults with FXS. This review focuses on the latest research in both FXS and in premutation involvement and their treatment.

Acknowledgments

This work was supported by the following NICHD grants HD036071, HD02274, NIDCR grant DE019583, NIA grant AG032115, NINDS grant NS062412, NIDA TL1DA024854, and 90DD0596 from the Health and Human Services Administration on Developmental Disabilities.

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Figure 1. Relation of FMRP and mRNA in FXS

Schematic representation of the function of *FMR1* gene and relationship among CGG repeat numbers, mRNA level and FMRP (fragile X mental retardation protein) production in normal, premutation and full mutation individuals.

Figure 2. The impact of FMRP on Protein Interactions in the cell

Cascade of reactions leading to LTD (Long Term Depression) and interactions with FMRP. The dotted lines are hypothetical and the T-shaped line is an inhibitory effect. 5' TOP mRNAs = 5' Terminal Oligo Pyrimidine mRNAs that code for proteins important for translation; Akt = protein kinase B (PKB); CYFIP = cytoplasmic interacting FMR1 protein; eIF4E = E4-like factor 4 – expressed, translation initiation factor, cap-binding; ERK = extracellular signal-regulated kinases, synonym for mitogen-activated protein kinase (MAPK); FMRP = fragile X mental retardation protein; Homer = scaffolding protein; MAPB1 = microtubule-associated protein 1B; MEK = (MAPK) mitogen-activated protein kinase; mGluR = metabotropic glutamate receptors; Mnk = MAP kinase integrating kinase;

mTOR = mammalian target of rapamycin/FK506 binding protein 12-rapamycin associated protein 1 (FRAP1); NMDA-R = ionotropic N-methyl-D-aspartic acid receptor; P-4EBP = phosphorylated emopamil binding protein (sterol isomerase); P70 S6K = p70 ribosomal S6 protein kinase; PAK3 = P21 (CDKN1A)-activated kinase 3; PDK = 3-phosphoinositide-dependant protein kinase; PI3K = class I phosphoinositide3-kinases; PIKE = PI 3-kinase enhancer, GTPase, enhances PI 3-kinase (PI3K) activity; PIP = prolactin-induced protein; PSD95 = post synaptic density protein; PTEN= phosphatase and tensin homolog; Rac/Ras = small guanosine triphosphatases (intracellular molecular switches)

Figure 3. The mGluR hypothesis of FXS (modified from Bear et al. 2004) [35]
 Reduction of synaptic strength in the dendritic tree in fragile X syndrome compared to normal. This figure was modified from Bear et al. 2004.

Figure 4. Biochemical Pathways of FXS: theoretical impact of mGluR5 Antagonists
mGluR1/5 pathways in normals and in those with fragile X syndrome after the use of an mGluR5 antagonist.