

Fragile X spectrum disorders

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Summary

The fragile X mental retardation 1 gene (*FMRI*), which codes for the fragile X mental retardation 1 protein (FMRP), is located at Xp27.3. The normal allele of the *FMRI* gene typically has 5 to 40 CGG repeats in the 5' untranslated region; abnormal alleles of dynamic mutations include the full mutation (> 200 CGG repeats), premutation (55-200 CGG repeats) and the gray zone mutation (45-54 CGG repeats). Premutation carriers are common in the general population with approximately 1 in 130-250 females and 1 in 250-810 males, whereas the full mutation and Fragile X syndrome (FXS) occur in approximately 1 in 4000 to 1 in 7000. *FMRI* mutations account for a variety of phenotypes including the most common monogenetic cause of inherited intellectual disability (ID) and autism (FXS), the most common genetic form of ovarian failure, the fragile X-associated primary ovarian insufficiency (FXPOI, premutation); and fragile X-associated tremor/ataxia syndrome (FXTAS, premutation). The premutation can also cause developmental problems including ASD and ADHD especially in boys and psychopathology including anxiety and depression in children and adults. Some premutation carriers can have a deficit of FMRP and some unmethylated full mutation individuals can have elevated *FMRI* mRNA that is considered a premutation problem. Therefore the term "Fragile X Spectrum Disorder" (FXSD) should be used to include the wide range of overlapping phenotypes observed in affected individuals with *FMRI* mutations. In this review we focus on the phenotypes and genotypes of children with FXSD.

Keywords: Fragile X syndrome, autism spectrum disorder, intellectual disability, developmental delay, premutation

1. Introduction

A variety of disorders are associated with mutations in the fragile X mental retardation 1 (*FMRI*) gene including fragile X syndrome (FXS) caused by a full mutation (> 200 CGG repeats in the 5' untranslated region of *FMRI* gene) leading to absence or deficiency of the FMR1 protein (FMRP) and premutation (55 to 200 CGG repeats) disorders characterized by elevation of *FMRI* mRNA 2 to 8 times normal. Although these 2 types of disorders are distinct in their phenotypes and molecular pathology, recent studies have demonstrated significant overlap that has been fertile areas for research. The term fragile X spectrum disorder (FXSD)

has been developed to emphasize the continuity of clinical involvement from the gray zone (45 to 54 repeats) throughout the premutation and into the full mutation range. *FMRI* mutations are dynamic in that they usually expand between generations particularly when passed on by a female to her children when it can expand from a premutation to a full mutation (1).

FXS was the first identified disorder in this spectrum and it was discovered in association with the fragile site of the X chromosome in two brothers in 1969 by Lubs and colleagues (2). In retrospect the first X-linked pedigree of intellectual disability (XLID) reported by Martin and Bell in 1949 turned out to be a fragile X pedigree when tested by the *FMRI* DNA test that was developed after the discovery of *FMRI* in 1991 (3,4). The fragile site was characterized by not only the CGG expansion to > 200 repeats, but also methylation of the cytosine bases leading to silencing of translation and little or no production of *FMRI* mRNA and FMRP. Since FMRP is a critical protein for regulation of translation

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for hundreds of mRNAs into their respective proteins, most of them involved with synaptic plasticity (5), the lack or severe deficiency of FMRP almost always leads to intellectual deficits as seen in males with FXS. In females with FXS the normal X produces FMRP so only 25% will have an IQ below 70 and an additional 50% will have an IQ in the borderline range (6).

Premutation disorders were first identified with the discovery of an increased incidence of early menopause (prior to the age of 40) in female carriers in 1991 (7). This has been confirmed by multiple investigators and has now been named fragile X-associated primary ovarian insufficiency (FXPOI) (8). Approximately 20% of female carriers have FXPOI, although the rate varies in a curvilinear fashion with CGG repeat number; the greatest prevalence of FXPOI is between 70 to 100 CGG repeats (9).

The next premutation disorder identified was the fragile X-associated tremor ataxia syndrome (10,11) seen initially in older male carriers (> 50 years) involving an intention tremor and cerebellar gait ataxia in addition to autonomic dysfunction, Parkinsonism, neuropathy, memory and executive function deficits followed by cognitive decline. This is a neurodegenerative disorder that occurs in approximately 40% of men and 16% of women with the premutation (12,13). FXTAS is hypothesized to be caused by mRNA toxicity from the elevated *FMR1* mRNA levels (14) leading to the production of pathognomonic inclusion formation in neurons and astrocytes throughout the CNS, peripheral nervous system and even in some organs such as the adrenals, heart and pancreas (15).

Currently there are numerous additional medical, neurological and psychiatric problems associated with the premutation both with and without FXTAS including depression (16), anxiety (17,18), migraines (19) hypertension (20), immune mediated disorders including fibromyalgia and hypothyroidism (21,22), sleep apnea (23), restless legs syndrome (RLS) (24), and neuropathy (25,26) often associated with chronic pain symptoms. Since the prevalence of the premutation is much higher (1 in 130-250 females and 1 in 250-810 males) (27) than those with the full mutation (1 in 4,000-7,000) the impact of multiple medical and neurological problems in premutation carriers is far more significant in the population than the full mutation (28,29). The association of other disorders in adults with the premutation led to multiple studies in children and here we present a review of the manifestations in children with FXSD.

2. Full mutation - Fragile X syndrome

The *FMR1* gene, which codes for the fragile X mental retardation protein (FMRP, a major negative translation regulator), is located at Xp27.3 from base

pair 146,993,469 to base pair 147,032,647 (GRCh37/hg19). The *FMR1* gene is highly expressed in the brain and testis (30). FXS is associated with a variety of neurological, cognitive and behavioral deficits, and less frequent dysmorphic features. Males with the full mutation and full methylation have little to no *FMR1* mRNA and little to no FMRP contributing to the clinical phenotype of FXS. The range of involvement in females is determined by the X-chromosome activation/inactivation ratio (the percentage of cells with active normal X chromosome) because this will determine how much FMRP is produced by the normal X chromosome depending on whether it is active or not.

2.1. Physical findings

The physical phenotype and dysmorphology of FXS include signs of a connective tissue disorder such as a long and narrow face, large and prominent ears, a high arched palate, hyperextensible finger joints, pectus excavatum, flat feet, soft skin and mitral valve prolapse. Other features include low muscle tone, and pubertal macroorchidism (31,32). Noteworthy approximately 30% of young children with FXS will not have obvious dysmorphic features; the physical features are associated with the FMRP deficits. The most evident effects of lower levels of FMRP in both males and females are prominent ears and hypermobility of the metacarpal-phalangeal (MP) joints (33,34). In males FMRP deficits are associated with a narrow face and large ears, while in females the FMRP deficits are associated with increased ear prominence and jaw length (35). In about 5-10% of children with FXS a Prader-Willi phenotype is observed including severe obesity, hyperphagia, hypogonadism and in some cases delayed puberty (36,37) (Figure 1). The reduced expression of the cytoplasmic interacting FMR1 protein



Figure 1. A female adolescent with FXS Prader-Willi-like phenotype.

gene (*CYFIP*, located at 15q11-13) is believed to be the cause of this phenotype (37).

2.2. Neurological disorders

In a national survey of caregivers of individuals with FXS (1,394 individuals), 14% of males and 6% of females were reported to have seizures (38). The seizures were easily treated, often partial and infrequent; however they were associated with more severe developmental and behavioral problems (38). Remarkably those with seizures are more likely to have ASD. The seizures may add to the severity of the phenotype because animal studies of early life seizures have shown that the FMRP leaves the dendrites and migrates to the perinuclear area during seizures, thereby depleting the dendrites of the regulatory effects of FMRP (39). Hypersensitivity to audiogenic stimuli and hyperarousal are also characteristics of children with FXS. These children have enhanced amplitude to sensory stimuli measured by electrodermal studies and a lack of habituation to repetitive stimuli (35). In addition, MEG studies also demonstrate an enhanced electromagnetic response to stimuli (36).

2.3. Cognition deficits

Male and female individuals with FXS present a wide range of learning disabilities in a context of normal, borderline IQ or mild to severe ID. The average IQ of males with the full mutation is 40 (40). Intellectual and developmental disability occurs in 85% of males and 25% of females. The level of FMRP correlates directly with IQ (41); males with the full mutation with unmethylated or only partially methylated alleles produce more FMRP than those with fully methylated alleles (35). The higher levels of FMRP explain the typically higher IQ (above 70) in high-functioning individuals with FXS. Similarly those individuals with "size-mosaicism" (full mutation plus premutation, gray zone or normal alleles) have a higher IQ than those without mosaicism. Therefore full mutation cells have a deficit of FMRP and the premutation cells produce an excess of *FMRI* mRNA, leading to mRNA toxicity but relatively normal levels of FMRP ("dual mutation effects", pathological involvement from two different mechanisms). Higher rates of psychotic thinking have been observed in individuals with this type of mosaicism leading to dual mutation effects (42). In females with FXS the normal X typically produces 25% to 50% of the normal FMRP level and these females have IQ scores that range from normal to moderate intellectual disability (6). Working and short-term memory (43), executive function (44), visual memory, visual-spatial processing (45) and verbal deficits are common in FXS (verbal comprehension and vocabulary) (46). Almost all males and approximately

30% of females with FXS have impaired speech (47).

In general, overall IQ declines with age in those with FXS because of the deficits in abstract reasoning which cannot keep up with the intellectual growth seen in typical children and adolescents (48). The adaptive skills also decline in FXS from adolescence into adulthood (49). This emphasizes the importance of early intervention with intensive behavioral/cognitive programs and targeted treatments early in life to improve or prevent cognitive decline.

2.4. Behavioral phenotype

FXS accounts for approximately 2-5% of all individuals diagnosed with FXS accounts for approximately 2-5% of all individuals diagnosed with ASD (50). In FXS about 60% of males have an ASD (51,52). About 80% of males and 30% of females with FXS have symptoms of attention deficit hyperactivity disorder (ADHD) (53). Sleep disturbances, such as difficulty falling asleep and/or interrupted sleep are also characteristic of individuals with FXS (54). Altered sleep patterns and dysregulated melatonin profiles were found in 13 boys with fragile X when compare with age-matched normal controls (55). Results showed greater variability in total sleep time, difficulty in sleep maintenance, and significantly greater nocturnal melatonin production in the boys with FXS.

A hallmark feature of FXS that can also occur in some premutation carriers is social anxiety. This behavior leads to the characteristic "Fragile X handshake"; where the individuals may shake the interviewer's hand or acknowledge his/her presence but will avoid eye contact until the interviewer looks away (56). Additional behavioral features include stereotypies such as hand-flapping and hand-biting, shyness, perseveration, mood instability, aggression and impaired speech (52). Cross-sectional analyses suggest that dimensions of problem behavior, anxiety, and hyperactivity are age-related; thus, age should serve as an important control variable in behavioral studies in FXS. Measures of anxiety, attention, and hyperactivity are highly associated with other behavior problems (29). There is evidence that autism scores decreased with time, particularly in communication and social aspects of adaptive behavior (57). However, emotional symptoms, behavioral difficulties, problems with peers and social behaviors may remain relatively stable over time (58). These trajectories may be associated with variations of FMRP, which in turn can be related to epigenetic changes, but there have been no large longitudinal studies that assess the molecular variations and behavior/cognitive correlations. Further longitudinal studies are necessary to assess the developmental trajectories of FXS across the lifetime and relate the outcomes to molecular and environmental factors.

2.5. Genotypes

The unstable dynamic *FMRI* mutation can result in "size-mosaicism", but cells of individuals who have only one size allele may also show different patterns of methylation (none, partial, and full methylation) referred as "methylation mosaicism". Some individuals may have the presence of three or more populations of cells with different size-alleles and methylation-patterns. Therefore, the complex molecular mechanism and multiple possibilities of genotypes results in the wide variety of clinical characteristics of individuals with FXS and may also relate to different responses to standard and targeted treatments but this has not been well studied (59).

2.6. Neurobiology

At the cellular level, FXS is associated with immature dendritic spine morphology (60,61). FMRP is an essential protein for synaptic development and plasticity because it is a key negative regulator mRNA translation and subsequent protein synthesis that can down-regulate and/or up-regulate their targets at the synapse (62). FMRP inhibits protein synthesis that is needed for internalizing the AMPA receptors leading to long term depression (LTD); thus without FMRP there is enhanced LTD in the hippocampus (63). The *Fmr1*-KO mouse shows enhanced protein translation and protein synthesis in the hippocampus (64), LTD is significantly increased and this leads to deficits in synaptic plasticity and weakening of synaptic connections (65). Protein synthesis promotes synaptic plasticity activation, which is thought to be mainly coordinated by the action of metabotropic glutamate receptors (mGluRs) (66). This is the basis of the "mGluR theory of fragile X syndrome" (63). The neurobiology and several symptoms of FXS were rescued when the mGluR heterozygous mouse was crossed with the *Fmr1*-KO mouse (63,67).

Currently there are many other pathophysiological mechanisms described that are thought to be the result of absence or low FMRP. The lack of FMRP can also up-regulate PI3K, an important signaling molecule downstream of the activation of mGluR (31). Recently Matic *et al.* (2014), showed a global down-regulation of the MAPK/ERK pathway and decrease in phosphorylation level of ERK1/2 in the murine *Fmr1* KO. However, others show an increase in this system in patient fibroblasts (68). A differential expression of many proteins involved in the p53 pathway, Wnt and calcium signaling was also found and led to postulate that calcium imbalance is part of pathophysiology of FXS (69). Although FMRP is mainly a negative regulator, there is evidence that it can up-regulate the translation of some mRNAs, such as those encoding GABAA receptor subunits ($\alpha 1$, $\alpha 3$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, and δ), which were significantly reduced in neocortex

and cerebellum of the *Fmr1*-KO mice (70). Other proteins required for GABA synthesis (Glutamate decarboxylase, GAD), transport (GABA transporter, GAT) and catabolism (GABA transaminase, GABA succinic semialdehyde) were also found to be reduced (71). A balanced GABA system is required for neuronal activation, network oscillations, neuronal synchrony and facilitation of movement and integration of information in many brain regions (72). The imbalance between the GABA and Glutamate systems is believed to contribute to the cognitive impairments, anxiety, hyperarousal, ASD, and epilepsy in children with FXS (73).

A novel FMRP target mRNA is the neuronal nitric oxide synthase (*NOS1* or *nNOS*) in mid-fetal human neocortex. FMRP was found to be a positive regulator of *NOS1* translation, controlling *NOS1* protein levels in a dose-dependent manner *in vitro* and *in vivo* (74), and the *NOS1* was severely reduced in the fetal and post-natal developing neocortex of FXS patients (74). The evidence of the multiple roles of nitric oxide (NO) in multiple neural processes such as synaptic developmental, retrograde signaling and synaptic plasticity (75-79) led to the hypothesis that the decrease expression of *NOS1* and secondary depletion of NO in the developing FXS brain may contribute to the neuropathology of FXS (80).

The absence of FMRP also affects the Brain Derived Neurotrophic Factor (BDNF) levels in early and late development in the murine hippocampus. In early development of the KO mouse brain, hippocampal expression of BDNF is increased compared to wild type (WT) (81,82), whereas by age 3-4 months, BDNF expression is reduced compared to the WT (82,83). The mechanism of regulation of BDNF remains to be described, but this evidence suggests dual FMRP effects in BDNF expression during brain development. FMRP may also positively regulate many other mRNAs including *SOD1*, *ASCL1*, *Kcnd2*, and *DLG4* (84-86). It is estimated that FMRP regulates the translation of about 4% of brain mRNAs (87,88). We have discussed the mechanisms of pathogenesis mediated by the absence of FMRP; however, the mechanism that causes the silencing of the *FMRI* gene by the full mutation remains uncertain. There are many targeted treatments that focus on these pathways to reestablish the normal neurobiology in the KO mouse and these have led to clinical trials of targeted treatments in patients with FXS.

2.7. *FMRI* silencing mechanism of the full mutation

It is intriguing that the premutation can lead to enhanced expression of the gene, whereas the full mutation leads to suppression of transcription. There are mechanisms that could explain the reduced transcription of the *FMRI* gene in the full mutation; these mechanisms can be divided in two groups: DNA-mediated and RNA-mediated (89). A model in which hairpin aggregation by the CGG repeats

results in the DeNovo methylation has been suggested because tridimensional CGG-structures can trigger their own methylation by DNA methyltransferases *in vitro* (90); another suggested DNA-mediated model involves repeat-binding transcription factors which in turn can aggregate other proteins and prevent transcription. This model was hypothesized from the existing evidence of a similar mechanism in mice where the pericentromeric repeats in mice are silenced by Pax3 and Pax9 hybridization and recruitment of H3K9 trimethylase and Suv39h1 (91) that finally inactivate these regions. The *FMR1* mRNA products are a variety of transcripts of different sizes and reverted sequences that result from a number of splicing sites and the transcription of both, the sense and anti-sense strands. Colak *et al.* (2014), suggested an RNA mediated mechanism of silencing, in which the *FMR1* gene is silenced through a hybridization of the complementary CGG-repeat track of the *FMR1* mRNA (92). Other RNA-mediated mechanisms have been suggested to involve the formation of RNA hairpins subtracts of the enzyme Dicer, RNA-DNA hybrids for chromatin compaction and promoter antisense-transcripts (89). The silencing mechanisms of *FMR1* are potential targets for drug therapy. Since the FMRP is a key transcription regulator of many neurobiological pathways, in theory targeted treatments to prevent the inactivation of the *FMR1* gene may lead to more normal FMRP levels and reestablish the function of many neurobiological systems. Therefore silencing gene modifiers could be more efficient, although more difficult to translate into patients than specific-system treatments, such as the mGluR5 antagonist and GABAA agonists.

3. Premutation allele

As previously mentioned in adults the premutation is associated with FXTAS, FXPOI and a variety of other medical/psychiatric problems. Recently the studies of children with the premutation have demonstrated that some carriers can demonstrate limited physical features of FXS in addition to psychological or developmental problems whereas most carriers do not show any symptoms.

3.1. Physical findings

Premutation carriers can present with facial dysmorphic features and the most common finding is prominent ears (89,90). Recently a study of premutation carriers found that 33% of postpubertal carrier males had macroorchidism (93). Those with macroorchidism had a lower verbal and full scale IQ and increased *FMR1* mRNA levels compared to those without macroorchidism (93). This suggests that about one third of individuals with the premutation have significantly lowered FMRP leading to their macroorchidism and

mildly lowered cognitive abilities. Premutation carriers can also have joint-laxity and smooth skin typical of those with FXS (94,95).

3.2. Neurological disorders

Chonchaiya *et al.* (2011) studied boys with the premutation and found an association between seizures, ASD, and ID. These problems are more common in premutation boys who present clinically compared to those who are identified through cascade testing. FXS children of premutation mothers with autoimmune disorders were found to have increased epilepsy and tics compared to children whose mothers did not have autoimmune problems (96).

3.3. Cognitive and behavioral phenotype

The cognitive effects of the premutation show variable results depending on the age of the carrier and whether they present as the proband or were identified through cascade testing. Not clinically referred children typically do not show differences compared to controls, particularly in girls (97). Proband carriers who presented clinically usually have cognitive deficits compared to controls (97,98). ADHD is increased in carriers compared to controls (97) and in adulthood these symptoms can persist or present as executive function deficits (34,99,100). Myers *et al.* (2001), in a small study of 14 children with the premutation found a trend towards lower performance IQ (101). Boys with the premutation have higher rates of ADHD symptoms, shyness, social deficits, autism spectrum disorder (98,102) and, less commonly, intellectual disability (ID) compared to controls. Many case reports of premutation involvement and ASD have been published. Clifford *et al.* (2007) reported seven males with the premutation; two were probands, and one of these had ASD (104). Goodlin-Jones *et al.* (2004), reported four premutation boys and two girls with ASD, and their levels of FMRP were significantly lower than normal (103). In the Farzin *et al.* (2006) study, there were 14 boys with the premutation whose parents sought medical attention for their sons' behavior problems (proband), 13 boys with the premutation diagnosed by cascade testing (non-proband), and 16 boys who were siblings without the premutation (controls). They found that 93% (13 of 14) of probands, 38% (6 of 13) of the non-proband and 13% (2 of 16) of the controls had ADHD. In addition 71% of probands (10 of 14) and 8% of non-proband (1 of 13) had ASD. In a screening study of individuals from families with FXS, about 14% of boys and 5% of girls with the premutation met diagnostic criteria for ASD (104). A web questionnaire of more than 1,000 families demonstrated a prevalence of autism or ASD of 13% in boys with the premutation and 1% in girls with the premutation (105).

Recently, the Rivera group at the MIND Institute (106) using a contrast-detection task found low-level visual processing deficits in infants with deficits in infants with FXS and with the premutation. In both groups of infants the contrast levels needed for detection of motion were significantly greater than those of typically developing infants. They concluded that early in life premutation infants can show visual or perhaps other deficits that are also observed in children with FXS.

Psychiatric problems in adults, including depression and anxiety, occur in about 40% of premutation carriers (14). Although initial studies of psychiatric disorders in premutation carriers hypothesized that the mood disorders found were associated with the difficulties of caring for a child with FXS, these problems can occur independently from having an affected child (17). In the life-time of individuals with FXTAS, 65% met the clinical criteria for a mood disorder according to the DSM-IV, remarkably for anxiety in 52% of the cases (17). It has been found that adult females have more problems with attention, hyperactivity (105), sleep problems (23), autistic behaviors such as rigidity (107), perseverance and aloofness (108) and language dysfunction (109) compared to controls.

3.4. Neurobiology

Hippocampal neurons with the premutation in culture (*in vitro*) showed reduced dendritic maturity with shorter dendritic lengths and fewer branches between 7 and 21 days compared with WT neurons (110). The premutation neurons had elevations of stress proteins and their mRNAs, including heat shock proteins (Hsp27 and Hsp70) and α B-crystallin. In addition premutation neuronal cultures die more easily in culture by 21 days compared with WT type neurons (110,111). Furthermore, altered embryonic neocortical development in the premutation mouse compared to WT has been reported (112). At 12 weeks early deficits in learning were observed in KO mice, the premutation mouse was unable to detect a change in the distance between two objects; and at 48 weeks, they could not detect a transposition of objects (113). This suggests that the premutation leads to a clear neuronal susceptibility that in addition to other genetic hits (93) or environmental toxicity (114) can result in a pathogenic neurobiology. Further studies are necessary to determine the neurobiology of affected individuals with the premutation.

3.5. Premutation genotypes

Initially *FMRI* premutation carriers were thought to have normal FMRP levels, however recent research findings suggest that carriers have elevated levels of mRNA due to increased transcription, but decreased level of FMRP because the translation is less efficient (95,103). As the premutation increases from 55 to 200,

the level of *FMRI* mRNA increases and the levels of FMRP begin to decline (115,116). Reduced *FMRI* translation is observed in adult individuals with large size premutation alleles (> 110 CGG repeats) and these individuals can have cognitive deficits. Also recent animal studies of the premutation mouse demonstrate lowered levels of FMRP in addition to elevated *FMRI*-mRNA in many brain areas, particularly the amygdala, hippocampus, and cortex, when compared with controls without the premutation (117).

The causative molecular mechanism of cognitive deficits and neurodevelopmental problems were thought to be related to silencing of the *FMRI* gene ("loss of function") and decreased amount of FMRP while the mechanisms involved in FXTAS and FXPOI are thought to be associated with abnormally increased levels of *FMRI* RNA ("gain of function") and RNA-toxicity. However recent evidence supports that both the FMRP deficits and elevated *FMRI* RNA in carriers are associated with amygdala dysfunction, which causes cognitive deficits, anxiety, autism spectrum disorders, social avoidance, and aggressive behavior.

There are at least 3 mechanisms that could explain the elevation of *FMRI* mRNA (89). One suggests that the observed increase of acetylated histones at the *FMRI* promoter (118) could increase the *FMRI* gene transcription. Second, the long tracts of CGG-repeats have been shown to exclude nucleosomes *in vitro* (119) and if this occurs *in vivo* it may increase the accessibility of transcription factors to the promoter. Third, the R-loops formed by the CGG-repeats (120,121) may lead to chromatin decondensation (122). The mechanism of *FMRI* mRNA-toxicity remains to be established, and there are at least 3 models proposed. The "sequestration" model which proposes that the RNA expanded CGG repeats are pathogenic by sequestering proteins, including Pura, Rm62, CUGBP1, hnRNP A2/B1, SAM68, and DROSHA-DGCR8 (123-127) that in turn alter the transcription of many other proteins. A second model, "RAN translation", represents non-canonical translation that results in expression of toxic polyglycine- and polyalanine-containing products (128,129). A third model, "antisense *FMRI* (ASFMR1) toxicity", involves the expression of antisense transcripts products (130). Mitochondrial abnormalities have also been found in FXS and premutation carriers. The mechanism of mitochondrial dysfunction is unknown but this mechanism is another cause of premutation and full mutation involvement (131,132).

4. Overlapping phenotypes, *FMRI* spectrum disorders

The overlap between premutation disorders and full mutation disorders occurs when the full mutation is partially or completely unmethylated or there is a high level of mosaicism in FXS. This puts those with FXS at

risk for FXTAS and other premutation problems. In fact there have been a handful of individuals with FXS who have developed FXTAS and these individuals are high functioning and have unmethylated alleles or mosaicism (133-136). Even in the midrange of CGG repeats in premutation carriers there may be mild deficits of FMRP leading to behavioral problems or psychiatric phenotypes (137).

Another area of overlap occurs in the gray zone (45-54 CGG repeats). The rate of *FMR1* gray zone expansions in the general population is variable, but large population studies report rates of 0.8% to 3.0% for repeat sizes between 41 and 54 (138-140). In 2006, it was recognized that gray zone expansion carriers can also present with premature ovarian insufficiency at a higher rate than in the general population (141,142). In a screening study in 2011 a higher rate of Parkinsonism was found in the gray zone mutation carriers compared to controls without the gray zone. There have also been reports of FXTAS in those with a gray zone (143,144) because elevated *FMR1* mRNA can also occur in this range (145). Other clinical associations with the gray zone in adults include anxiety (146) and cognitive decline (147). However other studies did not show this association (147-151). Pertinent to children, in 2000, a 5-year survey of boys who required special education showed an excess of gray zone expansions (152), however, this result has not been replicated (153). Further studies are necessary to study the association of the gray zone mutation and the mechanisms of disease in adults and children.

5. Conclusion

Clinicians need to know that those with an *FMR1* mutation are at risk for a wide range of neurodevelopmental and/or psychological disorders/neurological disease, referred as Fragile X Spectrum Disorders (Figure 2). It is also important to have a holistic model of understanding on how the phenotype is related to the number of CGG repeats and/or size-mosaicism, including epigenetic changes or methylation status (partial and full, as well as methylation mosaicism), genetic background

(gene modifiers and second genetic hits which can be protective or pathogenic) and environmental exposures (environmental changes, exposures to toxins, and social interactions "sociohome" among other factors).

Our understanding of FMRP deficits in the FXSD has been hampered by the limited technology available to assess quantitative FMRP levels. Although the immunocytochemical methodology demonstrated a strong correlation with IQ in those with a fragile X mutation (35,154), it was not sufficiently quantitative to show the remarkable variation that exists even in the normal population. This variation has been demonstrated by ELISA technology but the technique is difficult to replicate in subsequent samples (155). Newer techniques including the immunoassay utilizing time-resolved Forster's resonance energy transfer (156) and also the Luminex immunoassay (157). These techniques will lead to a new understanding of FMRP deficits not only in FXSD, but also in other neurodevelopmental/neuropsychiatric disorders. The recent publication of FMRP deficits in the brains of individuals with bipolar disorder, schizophrenia, depression and autism (156-158) has opened our eyes to the importance of FMRP outside of the FXSD population. Even more remarkable is the finding that the age of onset and overall IQ in those with schizophrenia is correlated with FMRP deficits in peripheral blood (159). The advances in treatments for FXS may also be helpful for premutation carriers with low FMRP and perhaps in other disorders with low FMRP such as ASD.

An area of overlap that is in need of research is the aging process in FXS because many patients experience cognitive decline and the cause is not known, although occult mosaicism leading to a FXTAS-like picture is possible (160). Older patients with FXS also have a high risk for Parkinson's disease and it is uncertain if this is also related to occult mosaicism (161). These are important considerations for children with FXS because they are raised by mothers with the premutation who may experience a premutation disorder that could influence the development of their offspring. These intergenerational influences require more study. Certainly the development of effective targeted treatments aim to have a significant effect on the ultimate outcome for those with FXSD.

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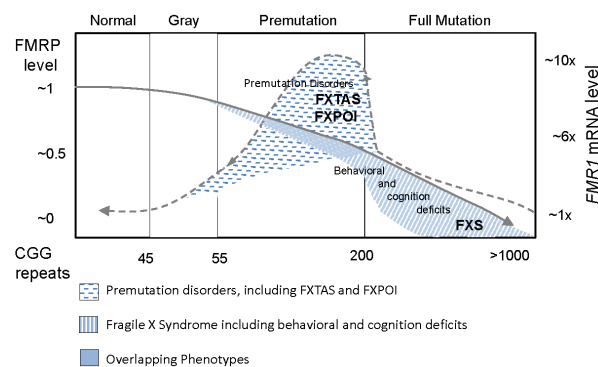


Figure 2. Overlapping phenotypes between FXS and premutation disorders. Dotted-line indicates FMR1 mRNA levels and solid-line indicates FMRP expression levels.

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