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REVIEW

Fragile X-associated disorders: a clinical overview

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Abstract Fragile X Syndrome (FraX) is the most common inherited cause of learning disability worldwide. FraX is an X-linked neuro-developmental disorder involving an unstable trinucleotide repeat expansion of cytosine guanine guanine (CGG). Individuals with the full mutation of FraX have >200 CGG repeats with premutation carriers having 55–200 CGG repeats. A wide spectrum of physical, behavioural, cognitive, psychiatric and medical problems have been associated with both full mutation and premutation carriers of FraX. In this review, we detail the clinical profile and examine the aetiology, epidemiology, neuropathology, neuroimaging findings and possible management strategies for individuals with both the full mutation and premutation of FraX.

Keywords Fragile X syndrome · MRI · Fragile X tremor ataxia syndrome (FXTAS)

Introduction

Fragile X syndrome (FraX) is the most common heritable form of intellectual disability (learning disability) worldwide. It is an X-linked neurodevelopmental disorder with recent data suggesting that FraX affects approximately 1 in 2,500 individuals [1, 2] with approximately equal rates in males and females [3]. The prevalence of the premutation carrier state is significantly higher with estimates of up to 1 in 251 males [1] and 1 in 100 females noted [4].

Significant variation exists in relation to prevalence data, however, with figures from Israel finding the frequency of the premutation carrier state in females to be present in approximately 1 in 130, with the full mutation present in 1 in 2,500 females [3–5]; data from Canada, noting the prevalence of premutation carriers to be 1 in 800 males and 1 in 260 females [6, 7] and data from Taiwan finding that the frequency of premutation male carriers was much lower, at approximately 1 in 1,670 [8].

As FraX is an X-linked neurodevelopmental disorder, females, due to the presence of one normal allele, have a reduction, but not a complete absence of FMRP, resulting in a less severe physical, cognitive and behavioural phenotype. The levels of FMRP in females with FraX are related to lyonisation, or the X activation ratio (one of two X chromosomes is randomly inactivated with a consequent variation in the proportion of active X chromosomes that have an affected allele) [9–11]. Individuals with the full mutation of FraX have a characteristic physical, cognitive and psychological profile, whilst some individuals who are premutation carriers may exhibit some of these features, albeit to a lesser degree.

FraX involves an unstable trinucleotide repeat expansion of cytosine guanine guanine (CGG) in the 5′ promoter end (Xq27.3) of the fragile X mental retardation 1 gene (*FMR1*) [12]. Individuals with the full mutation of FraX have >200 CGG repeats with premutation carriers having 55–200 CGG repeats. In the vast majority of cases, the CGG expansion of the *FMR1* gene is accompanied by methylation of the *FMR1* gene and loss of *FMR1* protein (FMRP) production [12, 13]. Absence of FMRP has primarily been associated with abnormal maturation of synaptic connectivity, which is argued to be the primary cause

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of the cognitive deficits frequently observed in FraX [14]. Approximately 15% of individuals with FraX display a mosaic pattern consisting of both premutation and full mutation alleles [15].

Clinical presentation

Full mutation FraX males (Table 1)

Individuals with FraX are classically characterized by cognitive and behavioural difficulties, facial dysmorphism, connective tissue anomalies and macro-orchidism. Macro-orchidism, although not specific for FraX, is the most consistent finding, present in 90% of boys by age 14 [16].

The physical phenotype of FraX males comprises a broad forehead, a long narrow face, large prominent ears, a high-arched palate, mitral valve prolapse, dermatoglyphic abnormalities including hyper-extendible finger joints, double-jointed thumbs, a single palmer crease and hand calluses and, as mentioned above, macro-orchidism [15–19]. The most common medical condition is epilepsy, which occurs in approximately 20% of individuals with FraX [20]; however several other medical conditions can

Table 1 Individuals with FraX—physical and psychological profile

	Full mutation FraX males	Full mutation FraX females	Premutation FraX males	Premutation FraX females
Physical symptoms	Broad forehead Long narrow face Large prominent ears Hyper-extendible finger joints Single palmer hand crease Macro-orchidism	Usually not evident or very subtle facial features	Subtle facial features (broad forehead, large ears)	Usually not evident or very subtle facial features
Cognitive symptoms	Moderate or severe intellectual disability Several executive function deficits Short-term memory deficits Impaired attention	Borderline intellectual disability (large range of IQ present) Executive function deficits	Normal intelligence Executive function deficits Short-term memory deficits	Normal intelligence Subtle executive function deficits
Psychiatric symptoms or disorders	Obsessionality ASD ADHD Anxiety disorders	Anxiety disorders Increased obsessionality ADHD Depression	Obsessionality Cognitive decline Mood disorders (principally depression) ASD ADHD	Increased emotionality Anxiety Depression
Behavioural symptoms	Social avoidance Aggression	Social anxiety Shyness Alcohol misuse	Alcohol and substance misuse	Alcohol misuse
Medical conditions	Seizures Strabismus Otitis media Gastrointestinal Problems Obesity Hypertension Mitral valve prolapse	Seizures	FXTAS	FXTAS POI Thyroid disorders Chronic muscle pain Hypertension Fibromyalgia Muscle pain

ASD autism spectrum disorder, *ADHD* attention deficit hyperactivity disorder; *FXTAS* fragile X-associated tremor ataxia syndrome, *POI* primary ovarian insufficiency

also occur including recurrent otitis media and strabismus, which require treatment to prevent hearing impairments and amblyopia [21].

Frequent behavioural characteristics in FraX males include poor eye contact, hand flapping, tactile defensiveness, impulsivity and a resistance to environmental change [22]. Indeed, individuals with FraX frequently demonstrate a need for sameness and often over-react (including aggressively) to novelty [23]. Gaze aversion, anxiety, hyperactivity, and social-interaction deficits are other common behavioural characteristics found in individuals with FraX [24]. Autism spectrum disorders (ASD) are significantly over-represented in individuals with FraX with 25–47% of individuals fulfilling diagnostic criteria [25–28], with this rate further increased when a diagnosis of pervasive developmental disorder not otherwise specified (PDDNOS) is included [29]. In many cases individuals with FraX display qualitative differences in autistic symptoms and behaviour compared to people with ASD alone [30]. For example, individuals with both FraX and co-morbid ASD demonstrate social interaction patterns suggestive of social aversion rather than a lack of interest in the social environment more typical of ASD [31], however individuals with FraX and co-morbid ASD may be indistinguishable from idiopathic ASD and may show a similar social disinterest.

Other psychological difficulties noted in FraX males include mood instability and aggression [32, 33]. Furthermore, anxiety, shyness and even mutism have been described in children with FraX [34] with 86% fulfilling diagnostic criteria for an anxiety disorder, social phobia and specific phobia being the most commonly diagnosed [35]. Attention deficit hyperactivity disorder (ADHD) is also over-represented in individuals with FraX [36].

The cognitive phenotype of males with FraX includes a moderate to severe intellectual disability, although approximately 11% have an intelligence quotient (IQ) in the mild intellectual disability range [37]; deficits in executive function, abstract reasoning and short-term memory (particularly verbal short-term memory); difficulties with attentional control and arithmetic and poor visuo-spatial processing [14, 24, 38–40]. Tasks requiring short-term memory for complex sequential information are particularly problematic for FraX males [39]. However, individuals with FraX perform relatively well on measures of visuo-perceptual recognition, constructional ability and vocabulary [38, 41], and perform competently at tasks that require short-term memory for simple, meaningful information [42]. Furthermore, the patterns of cognitive deficits for individuals with FraX are different from those of other IQ matched intellectually disabled individuals. For example, an IQ matched Down Syndrome group of boys demonstrated greater attentional control during tasks involving selective attention, divided attention and executive functioning [43].

In summary, FraX males display a relative strength for learning simple verbal and non-verbal tasks and can recall simple meaningful information, but display significant impairments on tasks which require the manipulation of internal representations or the retention of abstract non-sequential information [44, 45].

Full mutation FraX females (Table 1)

In females, as discussed in the introduction, there is a reduction but not a complete absence of FMRP. These lower levels of FMRP typically result in a less severe physical, cognitive and behavioural phenotypes in girls and women. However, some females have severe impairments that are equivalent to those seen in males.

The physical phenotype in females is milder than that in males, however many of the same physical symptoms have been described, including a high arched palate, prominent ears, a long narrow face, genu valgum and flat feet [46]. These findings are more prevalent in those with an intellectual disability [47]. Several other more rare findings include hyper-extendible finger joints, double-jointed thumbs, cleft palate, precocious puberty and connective tissue dysplasia [15, 47–49]. Some females present with no physical abnormalities but can have significant psychiatric, cognitive and executive function deficits.

Cognitive deficits, whilst less common in females with FraX, have been noted, with executive function deficits most prominent [50]. Approximately 25% of females with FraX have an intellectual disability (an IQ < 70); however, most females have an IQ in the borderline to low-normal range (IQ 70–90) [48]. IQ and other cognitive deficits in females with FraX have been shown to correlate with levels of FMRP [51–54]. Deficits in attention and increased rates of ADHD have also been reported [55].

Females with FraX have several behavioural problems with depression, the most common of these, with up to 50% of individuals having been shown to suffer either from depression or dysthymia [56, 57]. Anxiety disorders are also common and have been reported to occur in up to 77% of females with FraX [32]. Other difficulties include shyness, social anxiety, specific phobias, impulsivity and schizotypal features, which present as a pattern of interpersonal socialization deficits such as excessive social anxiety, odd behaviour, odd speech and inappropriate affect [32, 58, 59].

Individuals with premutation FraX (males and females) (Table 1)

Some male premutation carriers of FraX exhibit subtle facial characteristics similar to individuals with full mutation FraX [60, 61] and display a wide range of subtle executive function, memory, and language deficits compared to healthy controls, although there are no significant differences in IQ compared to the general population [62]. Most premutation carriers however have no physical features of FraX and thus appear normal. Other difficulties noted include increased obsessionality and alcohol and drug misuse or dependence [63]. Male premutation carriers have been reported to have an increased prevalence of intellectual disability, ADHD, and ASD [64–68]. Schizotypal personality features and avoidant personality disorders have also been noted [69].

Female premutation carriers of FraX usually have no facial features of FraX though some demonstrate a mild form of the physical phenotype of FraX [50, 70]. Increased emotional problems [71], with high rates of major depressive disorder [57, 69], and some anxiety disorders, in particular panic disorder and agoraphobia without panic disorder are present in *FMR1* premutation females [32, 57]. However, some anxiety disorders, including social phobia, specific phobias and post-traumatic disorders actually have been reported to not be increased in premutation female carriers of FraX in some but not all studies [57, 72]. Premutation carrier females of FraX have an increased risk of chronic muscle pain and thyroid disease—particularly hypothyroidism, which is associated with increased symptoms of depression and anxiety [73–75]. Elevated levels of follicle stimulating hormone are common [76], and ovarian insufficiency occurs in approximately 20% of individuals [77].

Two distinct adult onset medical disorders have been noted in premutation carriers of FraX: fragile X-associated tremor ataxia syndrome (FXTAS) and primary ovarian insufficiency (POI).

FXTAS is a progressive neurodegenerative disorder characterized by late-onset progressive cerebellar ataxia, intention tremor and cognitive decline in elderly male and female premutation carriers of FraX [78–80]. Other neurological findings that may occur with FXTAS include short-term memory loss, dementia, peripheral neuropathy, lower limb proximal muscle weakness, and autonomic dysfunction. FXTAS occurs in a subgroup of patients, with the prevalence of FXTAS estimated at 40–45% of males and 8–16% of females over 50 years of age with penetrance age-related [73, 78, 79, 81]. Whilst an increase in impulsivity and executive function deficits has been reported in male premutation carriers without FXTAS [82], no cognitive deficits have been observed in premutation carriers under 50 years of age [83].

Primary ovarian insufficiency (POI) is defined as the cessation of menses before the age of 40 and occurs in approximately 20% of premutation females with FraX [77, 84] compared to 1% of the general population [85]. POI is characterized by loss of oocytes, lack of folliculogenesis, reduced ovarian oestrogen production, elevated serum gonadotropin levels, amenorrhoea, and infertility in women before the age of 40.

In contrast to premutation carriers, women who carry full mutations do not have POI. The absence of ovarian dysfunction in full mutation females suggests that the lack of FMRP is not the cause of POI. Rather, it has been demonstrated that premutation carriers have an increased amount of mRNA in lymphocytes and neurons, but a normal quantity of FMRP. This combination of relatively high levels of an abnormal mRNA (which could trap some CGG binding proteins) and decreased levels of FMRP [4], may cause toxicity at blood lymphocytes, granulosa cells and the ovum [86], and consequently result in POI. Co-segregation of the premutation carriers of FraX and POI was first described in 1991.

Aetiology

Genetics

Several studies conducted since 1890 noted a 20–30% increased prevalence of males compared to females in institutions or schools for individuals with mental retardation (intellectual disability) [87]. This observation, and descriptions of families with an apparent X-linked inheritance of mental retardation, led to the suggestion in the late 1960s that mutations in genes on the X chromosome may be a significant factor accounting for this male predominance in intellectual disability. In 1969, Lubs [88] described a family with four "mentally retarded" males over three generations, all of whom showed a curious anomaly of the X chromosome upon cytogenetic examination, then termed marker X. After 1977, when the "fragile site" became more efficiently detectable under special conditions of karyotyping (culture of lymphocytes in low folate medium or in the presence of antifolates), FraX became increasingly recognized [89].

Fragile X mutations are unstable expansions of a CGG trinucleotide repeat, located in the first exon (non protein-coding) of the *FMR1* gene. Several disabling neuro-psychiatric and neurological conditions result from similar expanded trinucleotide repeats including Huntington's disease, myotonic dystrophy, Freidreich ataxia, spinal

palsy and bulbar palsy. The full mutation inactivates the expression of the *FMR1* gene, leading to the absence of FMRP [12, 13, 90]. Premutation carriers of FraX (expansions of 55–200 CGG repeats) have either normal levels or mild deficits in FMRP. Individuals with FraX may show a mosaic pattern: a mixture of premutation and full mutation alleles, most commonly caused by somatic instability of the full mutation in early embryogenesis leading to a retraction of the expanded CGG repeat [90]. This mosaic pattern occurs because the CGG repeat number expands to greater than 200 repeats in some cells (full mutation), whereas in other cells the repeat number fails to increase in size to 200 CGG repeats (premutation alleles). This mosaic pattern of FraX occurs in approximately 15% of cases [90], although figures as high as 40% have been noted [91]. A more rare type of mosaicism is "methylation mosaicism" and is associated with individuals having >200 CGG repeats (full mutation), but with incomplete methylation of the *FMR1* gene and thus a reduced amount of FMRP. Therefore, mosaic mutations allow the expression of some FMRP (usually at low levels), and in some cases have been associated with lesser degrees of intellectual disability [90].

The transition from premutation carrier status of FraX to the full mutation occurs through maternal transmission of the abnormal allele, with a probability (up to 100%) depending on the size of the premutation, with the smallest premutation alleles observed to undergo transition to the full mutation in the next generation recorded at 56 CGGs repeats [92]. It has been shown that an adenosine guanine guanine (AGG) interspersion stabilizes the repeats as instability of the *FMR1* gene is due to the length of uninterrupted CGGs. However, to our knowledge, technical difficulties have precluded the determination of this pattern of AGG interspersion in a diagnostic setting.

The *FMR1* gene codes for the cytoplasmic protein FMRP, which has RNA-binding properties. The *FMR1* gene spans approximately 40 kilobases (kb) of DNA and the protein encompasses 632 amino-acids, although several shorter forms have been observed in vivo as a result of alternative splicing of the 17 exons present [93, 94]. FMRP is abundant in neurons, particularly those in the cerebral cortex, cerebellum and hippocampus [94, 95], and is also present in other tissues, including spermatogonia and various epithelial tissues. FMRP has been detected in polyribosomes, particularly in the dendrites and contains functional domains allowing its transfer between the nucleus and the cytoplasm [96], and has been demonstrated to be involved in the shuttling of RNAs from the nucleus to the cytoplasm [97]. FMRP contains four RNA binding domains and binds to several mRNAs including its own mRNA [98]. FMRP associates with mRNAs and other proteins to form large messenger ribonucleoprotein (mRNP) complexes. These complexes are believed to participate in the transport, localization and translation of target mRNAs [99, 100]. The proteins comprising these mRNP complexes are largely unknown. However, certain candidate proteins exist, such as the autosomal homologs of FMRP, namely, the fragile X related proteins; fragile X mental retardation syndrome-related protein 1 (FXR1) and fragile X mental retardation syndrome-related protein 2 encoded by the *FXR1* and *FXR2* genes, respectively, and nucleolin, a protein very abundant in the nucleolus but also present in the cytoplasm [100]. All three proteins can shuttle RNAs between the nucleolus and cytoplasm. The FXR1 and FXR2 proteins have a high sequence similarity to FMRP, include similar functional domains identified in FMRP, such as RNA binding domains, and show a similar tissue distribution to that of FMRP [101]. Studies on FXR2 knockout mice demonstrate similar behavioural phenotypes to those in *FMR1* knockout mice, implicating a similar role for FXR2 in central nervous system function [101].

FMRP has also been suggested to be involved in synaptic plasticity. Synaptic plasticity adjusts the strength of synapses during global changes in neural activity, thereby stabilizing the overall activity of neural networks. The mechanisms for FMRP's putative involvement in synaptic plasticity include its regulation of mRNA translation and its effect on matrix metallo-proteinase-9 activity (MMP-9). The absence of FMRP in FraX is associated with a reduction in translation of mRNAs related to synaptic plasticity, in particular those specific dendritic mRNAs which encode cytoskeletal proteins and signal transduction molecules [102], and an increase in MMP-9 in the synapse [103]. MMP-9 is important for synaptic structure and plasticity. As will be described below, minocycline inhibits MMP-9 activity, and thus may potentially be a treatment for FraX.

Recent studies in Drosophila suggest that mRNA transport and translation are not only limited to dendrites but are also associated with axonal growth [93]. Alterations in the regulation of axonal growth and innervation in *FMR1* neurons may contribute to the dendritic and spine pathology characteristic of FraX [104].

Neurotransmitters/neuropeptides

In a genome-wide expression profiling study in fragile X knockout mice, 3 complementary DNAs (cDNAs) were found to be differentially expressed: GABA-A receptor subunit δ, Rho guanine exchange factor 12 and expressed sequence tag BU563433. Of these, the δ subunit of the GABA-A receptor has been postulated to have a putative role in the cognitive and behavioural phenotype of FraX [105]. GABA-A receptors are the predominant inhibitory receptors in the brain and have been implicated in anxiety, depression, epilepsy, sleep patterns, learning and memory,

all of which are affected to varying degrees in FraX [106]. Mouse and fly models of FraX have demonstrated a reduced expression of GABA-A receptors [107, 108], however no human studies (including neuropathology or PET imaging studies) have corroborated these findings to our knowledge to date.

Other amino acids, including glutamate (Glu) and *N*-acetyl aspartate (NAA) have also been implicated in the aetiology of FraX [109–112], with recent animal research particularly focusing on metabotrophic glutamate receptors (mGluR). We also look at evidence implicating matrix metallopeptidase 9 (MMP-9) with FraX.

FMRP is an RNA binding protein which modulates dendritic maturation and synaptic plasticity. One of the mechanisms postulated for this effect is its inhibition of mGluR1 and mGluR5 mediated mRNA translation in dendrites [113, 114]. Loss of FMRP may have several effects including long-term depression (LTD) of transmission at hippocampal synapses [115], which is associated with activity-guided synaptic elimination [116]. It has been suggested that neurological and psychiatric symptoms associated with FraX may be a consequence of an exaggerated responses to mGluR activation due to an absence of FMRP [115].

Mouse models have examined the mGluR5 antagonist 2-methyl-6-phenylethynyl-pyridine (MPEP). MPEP has been shown to block aberrant phenotypes in the *FMR1* mouse model of fragile X and has effectively reversed several phenotypes, including hyperactivity, seizures and pre-pulse inhibition deficits, and have shown remarkable improvements in synaptic plasticity and spine morphology [117]. Repetitive behaviours common in ASD, but also present in FraX, may also be ameliorated with MPEP [117]. A recent human study investigated AFQ056, a receptor subtype-selective inhibitor of mGluR5, in 30 male individuals with FraX and noted an improvement in behavioural symptoms of FraX as measured by the Aberrant Behaviour Checklist-Community Edition (ABC-C) [118]. Another putative treatment for FraX via this mechanism is Fenobam, a high potency selective mGluR5 antagonist. Fenobam was previously investigated as an anxiolytic in a number of phase II studies in the early 1980s. However, a number of subjects suffered neurological and psychiatric symptoms including vertigo, paraesthesias, hallucinations and insomnia [119, 120]. A more recent open label, single dose study demonstrated no such adverse effects [121].

As described above, the absence of FMRP has been associated with higher levels of matrix MMP-9 in the brain [122]. Minocycline, a broad spectrum tetracycline antibiotic, inhibits MMP-9 activity, and in *FMR1* knockout mice has been shown to alleviate both synaptic and behavioural abnormalities [122]. An open-label add-on pilot trial evaluating the safety and efficacy of minocycline in treating behavioural abnormalities in humans with FraX demonstrated functional benefits to individuals with FraX, including an improvement in language and behaviours, and was also well-tolerated, with loss of appetite the only significant adverse effect noted [123]. These initial findings are consistent with the *FMR1* knockout mouse model findings, suggesting that minocycline may modify underlying neural defects, which could account for some of the behavioural abnormalities found in individuals with FraX [122].

Neuropathology findings

Post-mortem studies in people with FraX have reported dendritic spines abnormalities; characterized by spines that are longer, thinner and more tortuous in shape and lacking the typical "mushroom shape" associated with mature dendritic spines [124–127]. While dendritic spine anomalies are present in several neuropsychiatric conditions associated with intellectual disability, they are increased in density in FraX, which appears to be unique to this condition [128]. FMRP has been suggested to be involved in dendritic maturation and this is supported by reports that *FMR1* knockout mice have a significant decrease in the number and function of hippocampal neuronal synapses [127, 129, 130]. As described above, the dendritic spine dysgenesis found in FraX is typical of the morphology of the "immature brain" prior to synaptic elimination.

Eosinophilic, ubiquitin-positive inclusion bodies are the principal neuropathological finding in FXTAS and are located in the nuclei of neurons and astrocytes throughout the brain and the spinal column [131]. These inclusions are tau-negative and alpha-synuclein negative and contain *FMR1* mRNA [132].

Further neuropathology findings in individuals with FXTAS include a patchy loss of axons throughout the brain, spongiosis of the middle cerebellar peduncles and loss of purkinje cells [131, 133].

Neuroimaging findings

Full mutation FraX

Structural magnetic resonance imaging (MRI) studies have noted several morphological differences in brain structure in individuals with FraX compared to healthy controls. The most replicated findings to date include increased volume of the caudate nucleus [134–140] and reduced volume of the cerebellum [136, 141, 142] compared to both healthy comparison groups and those with ASD. Other findings

noted in some studies include increased volume of the lateral ventricles [135, 143, 144]; hippocampus [145–147]; parietal lobes [136, 139], and brainstem [139] and reduced volume of the cerebellar vermis [141].

The caudate nuclei are involved with regulation of impulse control and attention, and these neuroimaging findings may help explain the impulse control and attentional deficits frequently reported in individuals with FraX [33, 148]. This proposal has been supported by a recent report of altered fMRI activation in the right caudate of individuals with FraX engaging in an attention (go–no go) task [149]. The findings of cerebellar abnormalities may also explain some of the cognitive phenotype found in individuals with FraX.

The cerebellum is important in many higher order functions commonly impaired in individuals with FraX—e.g. attention [150], social interaction [151] and executive functioning [152] and has been found to be reduced in volume in ASD [153], which as mentioned above is increased in prevalence in individuals with FraX [24].

Premutations carriers of FraX

Most neuroimaging studies in premutation carriers of FraX are in individuals who have also been diagnosed with FXTAS. Studies in FXTAS have demonstrated reduced cerebral and cerebellar volume (males > females) compared to controls, with particular volume reductions in the middle cerebellar peduncles, the caudate nucleus and the parietal lobes [62, 153–156]. These regions have been implicated in motor and cognitive functions including coordination and attention [78], frequent difficulties in individuals with FXTAS. In premutation carriers of FraX without FXTAS, reduced volume in a number of regions including the amygdala–hippocampal complex bilaterally [62], the left hippocampus [156] and the left thalamus has been documented [62, 157], with a negative correlation between total hippocampal volume and anxiety in female carriers also reported [158, 159]. No difference in hippocampal or amygdala volumes between premutation carriers and normal controls has however also been observed [157, 158]. Although some studies have noted increased volume of the hippocampus in FraX individuals [145–147] and reduced hippocampal volume in premutation carriers of FraX [62, 157]; one study noted no difference between premutation and full mutation FraX individuals in relation to hippocampal volume [147].

Abnormalities in the hippocampus and amygdala have been suggested by findings in a number of fMRI studies in premutation carriers of FraX without FXTAS. Reduced hippocampal activation during a memory recall task [157] and reduced amygdala activation during a perceptual task when viewing fearful faces [158] have been observed.

Management

Screening

Screening individuals for any neuro-psychiatric disorder is a sensitive issue; however, given the recent evidence of the high frequency of FraX (both full mutation and pre-mutation carriers) in the population, it perhaps should be considered [14]. For example, an anonymous population screening, performed in Canada in 1995 on 10,624 females led to the detection of 41 previously undiagnosed premutation carriers of FraX (an incidence of 1 in 250) [6]. FraX is detected using DNA analysis: standardized Southern blot and polymerase chain reaction (PCR) analyses are performed followed by *FMR1* specific probe hybridization [160]. The CGG repeat number is calculated from the Southern blot autoradiogram images. FMRP levels can be ascertained by calculating the percentage of peripheral lymphocytes containing FMRP using immuno-staining techniques [161]. More recently, a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) has also been developed to measuring FMRP levels in peripheral blood lymphocytes [162].

Prenatal diagnosis can be performed to determine if a foetus has inherited the full mutation; once a premutation or full mutation carrier of FraX has been identified in the mother, although such testing should only be performed in conjunction with appropriate counselling for the family concerned. The sensitivity of prenatal testing is approximately 99%, although in very rare cases, FraX may result from point mutations, deletions in the *FMR1* FRAX-A gene or go undetected due to mosaicism.

Screening for *FMR1* mutations has been a topic of consideration since the *FMR1* gene was first identified. Advances in our understanding of the molecular basis of FraX and advances in genetic testing methods have elicited new prospects for identifying a greater number of individuals at risk for the disorder or at risk of transmitting the disorder [163]. McConkie-Rosell et al. [163] have suggested that individuals suitable for screening include children, grandchildren, or siblings with intellectual disability, autism, and social/behavioral, or learning disorders; daughters or female relatives with infertility, premature menopause, or both; and family members with tremor, ataxia or other neurological (neuropathy, multiple sclerosis), and/or psychiatric problems (anxiety disorders, depression, dementia, cognitive decline). This, however, is a very wide grouping of individuals, although there does appear to be merit in screening some of these individuals at least. We believe that there are two general types of circumstance in which fragile X testing should be considered: a clinical presentation suggestive of fragile X syndrome, including FXTAS or POI, or where there is a risk of

inheritance of FraX due to a family history of FraX or intellectual disability of unknown cause.

In relation to clinical presentation; genetic testing for fragile X should be considered in children with developmental delay including specific speech, language or motor delay, children with a diagnosis of intellectual disability of unknown aetiology and ASD. Testing children with borderline cognitive deficits leading to a diagnosis of FraX can be used to improve educational strategies for the child and help their families better understand their child's' difficulties [15]. Individuals over 50 years old with a recent onset of tremors, balance disorders, or Parkinsonian-like findings without a diagnosis would also appear to be a group that should be tested for FXTAS. Similarly, we believe that women with unexplained infertility or POI should also be considered for testing [164].

Screening should also be considered in individuals with a family history of fragile X to determine if they may be carriers and at risk of transmitting it to future generations, and in individuals with a family history of mental retardation or autism of unknown cause.

The recent findings of significant phenotypes in premutation carriers of FraX have significant consequences for genetic counselling. Furthermore, in females, unlike males who are premutation carriers of FraX, the number of CGG repeats can increase to a full mutation when passed on to offspring.

Further epidemiological studies are required to better estimate fragile X allele frequencies for all racial and ethnic groups, and greater knowledge is needed regarding the penetrance of *FMR1* associated disorders, FXTAS and fragile X-associated POI, in order to provide anticipatory guidance and to assist with the development of genetic counseling protocols [163].

Pharmacotherapy (see Table 2)

There are no pharmacological treatments presently available that ameliorate the cognitive deficits in FraX. However, a variety of agents have been utilized for the behavioural and psychological difficulties, although a paucity of controlled studies exist that formally measure their effectiveness [165]. Of those that are present, methylphenidate, dexamphetamine and L-acetylcarnitine have demonstrated some benefit for attention and behavioural difficulties [166, 167]. Anticonvulsants used to treat seizures may also improve autistic features, mood instability and tantrums [79], whilst antidepressant agents such as selective serotonin re-uptake inhibitors (SSRIs) may improve depression and anxiety disorders.

Future pharmaco-therapeutic strategies for FraX may focus on GABA and Glu, with evidence that the mGluR5 antagonist, 2-methyl-6-phenylethynyl-pyridine (MPEP), abolishes the audiogenic seizure phenotype in *FMR1* knock-out mice [168], and decreases the mushroom body defects (fused β-lobes) [169]. These findings have been replicated in multiple animal models and with many phenotypes and have led to several human phase II trials that are on-going. As discussed above, a recent study investigated AFQ056, a receptor subtype-selective inhibitor of mGluR5, in 30 male individuals with FraX noted an

Table 2 Pharmaco-therapeutic approaches in FraX and areas of potential benefit

	Behavioural difficulties	Attention	Repetitive behaviours	Hyperactivity	Cognition	Mood instability/ depression	Anxiety
Stimulants	+	+	+	+	−	−	−
Methylphenidate							
L-acetylcarnitine							
Antidepressants	−	−	+	−	−	+	+
SSRIs							
TCAs							
Anticonvulsants	+	−	−	−/+	−	+	−
Sodium valproate							
Carbamazepine							
mGluR5 antagonists	+	+	+	+	−	−	−
MPEP							
AFQ056							
Fenobam							
Minocycline	+	−	−	−	+	−	−

+ potential benefit, − no evidence for benefit, −/+ equivocal evidence for benefit, *SSRIs* selective serotonin reuptake inhibitors, *TCAs* tricyclic antidepressants, *mGluR* metabotrophic glutamate receptors, *MPEP* 2-methyl-6-phenylethynyl-pyridine

improvement in behavioural symptoms of FraX [118]. Fenobam, a high potency selective mGluR5 antagonist is a further putative treatment option given recent evidence of a good safety profile [121]. Furthermore, minocycline, the broad spectrum tetracycline antibiotic, has also shown some promise in an initial open-label study for improving behavioural difficulties in individuals with FraX [123].

Psychological/environmental approaches

Individuals with FraX have shown benefits from non-pharmacological interventions such as speech, occupational and sensory integration therapies. Research in *FMR1* knock-out mice has shown that an enriched environment can rescue many behavioural and neuronal abnormalities [170]. This suggests that early and intensive psychological and environmental interventions may substantially benefit the development of an individual with FraX.

Conclusions

FraX is a common genetic disorder resulting from a single-gene mutation on the X chromosome and is associated with a wide spectrum of physical, behavioural, cognitive, psychiatric and medical problems, with males more severely affected than females. Over the last decade our understanding of FraX has considerably increased, with conditions such as FXTAS and POI now known to affect premutation carriers of the condition. Early diagnosis of FraX is important to allow the introduction of appropriate educational and clinical interventions. Whilst there are few controlled trials to guide management to date, several medications can ameliorate medical, psychiatric and behavioural difficulties associated with FraX and can improve an individual's quality of life. Future treatments should possibly be aimed at targeting specific synaptic mechanisms affected in FraX.

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