Fragile X Syndrome

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Abstract

Fragile X syndrome (FXS), also known as Martin-Bell syndrome, is the most common inherited cause of intellectual disability (ID), and it is the most common known single-gene cause of autism or autism spectrum disorders (ASDs). It is caused by a trinucleotide repeat (CGG) expansion at the 5′ noncoding region of the fragile X mental retardation 1 (*FMR1*) gene located on the long arm of the X chromosome at band Xq27.3. Large expansions (>200 CGG repeats) cause hypermethylation and silencing of the gene, leading to a loss of the gene product, fragile X mental retardation protein (FMRP). The resulting phenotype is complex and consists of cognitive impairments, difficulties with emotional and behavioral regulation, and certain physical characteristics as well, all elaborated upon later in this chapter.

However, even smaller expansions (between 55 and 200 repeats) within the premutation range with normal or close-to-normal levels of FMRP can also result in clinical complications of its own. This is due in large part to the increased transcription of *FMR1* mRNA seen in premutation carriers, which can be toxic to cells, particularly neurons and astrocytes. The resulting premutation-associated disorders are discussed at length in the second part of this chapter.

Finally, pharmacological interventions that are still being studied are discussed. These pharmaceuticals, including mGluR5 antagonists and GABA agonists, seek to restore the imbalance of neurotransmission in FXS and have led to some promising preliminary results in mitigating or even reversing the neurobiological abnormalities caused by the loss of FMRP.

Keywords

Anxiety • Attention deficit/hyperactivity disorder (ADHD) • Autism • CGG repeats • Fragile X syndrome (FXS) • Gamma-aminobutyric acid (GABA) • Hypotonia • Intellectual disability • Matrix metalloproteinase 9 (MMP9) • Methylation • Minocycline • RNA toxicity • Seizures • Sleep disturbances • Tremor/ataxia syndrome

Abbreviations

FM	Full mutation
FMR1	Fragile X mental retardation 1 (gene)
FMRP	Fragile X mental retardation protein
FXPOI	Fragile X-associated primary ovarian insufficiency
FXS	Fragile X syndrome
FXTAS	Fragile X-associated tremor/ataxia syndrome
ID	Intellectual disability
PM	Premutation

Brief History

It was noticed as early as the late 1800s that males were disproportionately represented among the intellectually disabled population. However, this was presumed to be merely an artifact of societal norms, such as the higher intellectual

expectations for males at the time. It was not until the mid-to-late 1900s that genetic underpinnings were given serious consideration. In 1943, J. Purdon Martin and Julia Bell first reported a phenotype consistent with FXS after studying a large family pedigree containing multiple males with intellectual disability. This is considered the first example of X-linked mental retardation (XLMR), and they were later diagnosed as having FXS. In 1969, Lubs reported a cytogenetic abnormality that he called the marker X chromosome, which was later named the fragile X chromosome because there was a fragile site or a break on the bottom of the X chromosome, in two brothers with ID. This fragile site was later found to occur only with folate-deficient tissue culture media by Grant Sutherland in Australia in 1977. Subsequently, many individuals were diagnosed with FXS through cytogenetic testing, demonstrating the fragile site on the X chromosome.

The gene mutation that leads to FXS was not discovered until 1991 by an international consortium. This gene at Xq27.3 was named the fragile X mental retardation 1 (*FMR1*) gene, and it has a trinucleotide (CGG) repeat sequence at the 5′ untranslated region, with most people in the general population typically having about 29–30 CGG repeats, with a range of about 5–44. The fragile X mutation is an expansion of this CGG sequence with the full mutation defined as >200 repeats, subsequent methylation leading to a lack of gene transcription, and a lack of the *FMR1* protein, FMRP. It is the deficiency or absence of FMRP that causes FXS. Those who have between 55 and 200 repeats are considered to be in the premutation range, and while these individuals themselves are not typically affected by FXS, the gene is at high risk of expanding into the full mutation range when it is passed on by a female to the next generation. Occasionally, individuals with the premutation have mild deficits of FMRP, particularly in the 120–200 CGG repeat range, and they then demonstrate mild features of FXS. Those with the premutation may have other medical problems, however, related to having elevated levels of the *FMR1* mRNA levels, as discussed later in this chapter. There is an additional intermediate allele category named the gray zone, ranging from 45 to 54 repeats, in between the normal and premutation categories. Alleles in this range can sometimes expand into a premutation in the next generation, but not directly into a full mutation when passed on by a female.

Around the time of the discovery of this gene, it was assumed that those in the premutation range were asymptomatic, and the only concern was the possibility of expansion to a full mutation in the next generation. However, a wealth of evidence has accumulated that indicates the existence of a subtler range of clinical involvement, even in those who merely carry the premutation form of the gene. Reports of early menopause and ovarian dysfunction related to the *FMR1* gene began to appear in 1991, first by Cronister et al. and then replicated by many others. By the late 1990s, it became well established that this phenomenon was real and singular to the premutation, and not the full mutation. This was later coined fragile X-associated primary ovarian insufficiency (FXPOI). Then, at around the turn of the century, Hagerman and colleagues discovered the existence of the fragile X-associated tremor ataxia syndrome (FXTAS), a neurodegenerative disorder in older premutation carriers, mostly male, that results in a host of neurological and cognitive issues, to be expanded upon later in this chapter. Since that point in time,

premutation carriers have become an item of intense interest in research, and a clinical picture involving a range of neurological, endocrinological, and psychiatric symptoms is emerging.

In the past few years, research has also heavily focused on exploring and reversing the neurobiological aberrations caused by the *FMR1* gene. Knowledge in this area of research has increased exponentially ever since the discovery of the gene in 1991, particularly after the advent of the *FMR1* knockout mouse model of fragile X, which has a phenotype very similar to that of humans. Studies using this mouse model have revealed a number of promising molecular targets for pharmacological remediation, which has caused excitement within the fragile X community for prospects of early pharmacological intervention to reduce or arrest the neurodevelopmental abnormalities in FXS.

Fragile X-Associated Disorders Are Caused by CGG Repeat Expansion

The Full Mutation (>200 CGG Repeats) Is Caused by Methylation-Induced Gene Silencing

The full mutation, defined as over 200 CGG repeats, causes FXS. Methylation usually sets in at around 200 repeats, resulting in silencing of the *FMR1* gene and the cessation of production of mRNA and FMRP. FMRP is an RNA-binding protein that transports mRNA to the synapse where it controls translation of its target mRNAs, usually acting as a translational repressor until its inhibitory effects are released by an appropriate stimulus. In this manner, FMRP regulates hundreds of proteins, and in its absence, there is severe and unchecked upregulation of protein production in the central nervous system (CNS). FMRP is therefore essential for proper brain development and plays a vital role in synaptogenesis as well as synaptic plasticity, pruning, and maturation.

It is the deficiency of FMRP that causes the physical, behavioral, and cognitive features of FXS. The majority of males with the full mutation (FM) exhibit mild to severe cognitive deficits and the characteristic physical phenotypes of a long, narrow face; large ears; and macroorchidism (beginning in puberty). Females with the FM are typically less affected than their male counterparts since they have an extra X chromosome to buffer the debilitating effects of the mutation. Those with a higher activation ratio, the percentage of cells expressing the normal X chromosome, tend to have a normal IQ, although emotional problems are common.

Some individuals with the FM in some cells also carry the premutation in other cells. Such individuals are referred to as mosaic. There are two types of mosaicism in FXS. The aforementioned is known as size mosaicism, resulting from cell-to-cell variation in length of CGG repeats. Other individuals with the FM contain some cells that are partially or completely unmethylated. This is known as methylation mosaicism, which can be common among individuals hovering around the lower end of

the full mutation (between 200 and 230 CGG repeats). However, as complete methylation sets in beyond this point, there becomes no significant correlation between the degree of phenotypic manifestation and the CGG repeat length. Both size and methylation mosaics have some cells that actively produce mRNA and FMRP. Subsequently, although these individuals tend to have a better prognosis in childhood and young adult life, they also tend to have elevated mRNA, which, as discussed below, may put them at risk for premutation-related problems, such as neurological disease with aging. However, to date, there has been no reported case of FXTAS in FXS.

The Premutation (Between 55 and 200 CGG Repeats) Has Elevated Levels of mRNA

Individuals with premutation (PM) alleles, which contain between 55 and 200 CGG repeats, generally produce normal to slightly low levels of FMRP. Therefore, PM carriers were once thought to be largely unaffected by the gene mutation since they do not usually exhibit the overt cognitive, physical, or behavioral features of FXS. However, further research into the matter has revealed subtler issues along the FX spectrum that are sometimes present, such as ADHD, anxiety, emotional and psychiatric problems, and some physical features as well. A subgroup of PM carriers may also exhibit more severe problems reminiscent of FXS, such as ASD or cognitive impairment. Furthermore, the premutation state is associated with its own distinctive pathologies too, such as FXTAS and FXPOI.

With normal or close-to-normal levels of FMRP in most PM carriers, some other molecular variable must also be present to account for the range and extent of symptomatology in PM carriers. It would be natural to assume that there would also be normal or close-to-normal levels of *FMR1* mRNA in direct proportion to FMRP levels. However, contrary to expectation, there is actually an elevation of mRNA levels, about two to eight times above normal, as discovered by Tassone et al. in 2001. Moreover, this elevation is roughly proportional to the CGG repeat size and is paradoxically more pronounced in the upper end of the premutation where FMRP tends to be lowest. There is an apparent deficit of translational efficacy of *FMR1* mRNA with increasing CGG repeat size, causing reduced translation of FMRP. It is hypothesized that a negative feedback loop exists between FMRP and the *FMR1* promoter region, such that the reduced levels of the former stimulate the latter to upregulate transcription of mRNA as a compensatory mechanism to normalize levels of FMRP, though this mechanism is still not able to fully account for the FMRP reductions in some carriers. This deficit in translational efficacy seems to be directly proportional to CGG repeat length, resulting in higher and higher levels of mRNA with increasing CGG repeat size until eventually methylation sets in and silences the gene completely, reducing mRNA levels to close to zero and creating a mechanistic switch that ushers in the molecular pathology related to the full mutation.

This observed phenomenon has led to the gain-of-function RNA toxicity model that is the prevailing model behind premutation involvement. This hypothesis posits that the elevated levels of *FMR1* mRNA create a toxic environment for cells by attracting RNA-binding and other proteins, sequestering them away into aggregates of mRNA and eventually into intranuclear inclusion bodies and preventing their normal function from being expressed. These inclusions have been discovered in the brains of patients with FXTAS upon postmortem neuropathological examination and have been stained positive for *FMR1* mRNA and ubiquitin. This sequestration causes dysregulation of a number of different proteins in the CNS, leading to enhanced cell death and the eventual neurodegeneration and white matter disease seen in patients with FXTAS. It is hypothesized that this RNA toxicity may, at least to some extent, underlie much of the other involvement seen in premutation carriers as well, including the neurodevelopmental problems, psychiatric problems, social deficits, and FXPOI.

Full Mutation Transmission Occurs from Mother to Child

Within the normal allele category (5–44 repeats), transmission from generation to generation is usually stable without a change in CGG repeat size. FM alleles, therefore, are derived from PM alleles, which are very unstable and prone to expansion, or from other FM alleles. The FM is transmitted strictly matrilineally, and an FM or PM mother has a 50 % chance of passing on the mutated allele to each of her offspring, similar to any other X-linked gene. An FM mother will pass on the FM allele. A PM mother may pass on another PM allele, but she is also at high risk for passing on an FM allele, but in either case the allele will usually expand. The risk of FM transmission increases in proportion to the mother's CGG repeat size. A minority of PM alleles with under 60 repeats will expand to an FM, with the risk slowly increasing until about 100 repeats, after which point all of the mutated alleles will expand to an FM when passed on by a female.

In contrast, the paternal allele is relatively stable and does not usually expand significantly in subsequent generations. In fact, contractions (wherein the repeat size becomes smaller in subsequent generations) are not uncommon, and expansion to an FM almost never occurs, presumably due to the strong selection against the FM allele in the sperm line. Therefore, all the daughters (and none of the sons) of a PM father will inherit the PM allele, but none will inherit an FM.

To date, there has been no evidence of a single-generation expansion from a gray-zone allele to an FM allele, though sometimes it can expand into a PM allele. In general though, gray-zone alleles are relatively stable and in many families may not change even across several generations and may even contract. The smallest allele to ever expand to an FM in one generation had 56 repeats, which is at the bottom of the PM range.

It is believed that one of the leading causes of gene instability in the PM and FM alleles is the absence of AGG anchors. In normal alleles (<45 repeats), there is

usually a cytosine to adenine transversion every 9 or 10 CGG repeats or so. This creates an AGG interruption within the CGG repeat strand, which acts as an anchor to help stabilize the structure, perhaps by impeding the formation of hairpin loops in the DNA caused by long-repeat CGG tracts. These hairpin loops otherwise tend to promote replication slippage, thereby causing CGG repeat expansions. Typically, a premutation-range allele will have only two or three anchors and long stretches of pure CGG repeats. When the anchors are lost or reduced relative to the CGG repeats, there is more instability and therefore a greater chance of expansion from one generation to the next when passed on by a female.

Epidemiological Studies Reveal Widespread Involvement

Previous studies of FXS prevalence based on population projections from cohorts of intellectually disabled (ID) individuals have reported a frequency of approximately 1/3,000 to 1/4,000 for males and 1/6,000 to 1/8,000 for females. However, such estimates are imprecise because they were not based on population genotyping and were extrapolated from patients who presented clinically with ID. The prevalence of females with the full mutation is spuriously low because many females with the full mutation have borderline or normal IQ and may not present clinically, although they may have behavioral or learning problems associated with FXS. When taking into account the entire spectrum of FXS, which includes not only ID, but also behavioral, emotional, social, and academic difficulties, some of which may be subtle, the true prevalence is similar to the male prevalence, about 1 in 3,600.

Ultimately, a true estimation of FXS prevalence would require large-scale newborn or general population screening, some of which have taken place. So far such studies have demonstrated a prevalence of the premutation at 1 in 130–260 for females and 1 in 250–810 in males. These numbers vary depending on the population studied, presumably related to regional founder effects. Full mutation prevalence is harder to determine due to the much larger sample sizes necessary to reach statistically significant estimates. Until then, estimates extrapolated from clinical populations, such as described above, will suffice, and these estimates are fairly close to mathematically calculated estimates (~1/2,500) based on female premutation frequency and the observed rate of PM to FM transmission.

The frequency of gray-zone alleles in most populations is about 2–4 %, depending on where the allele cutoffs are defined (45–54 repeats or 40–54 repeats). The gray zone is being studied now for clinical involvement, and currently the rate of POI is twice that seen in the general population. There is also preliminary evidence of a higher rate of parkinsonian symptoms in those with a gray-zone allele. Therefore, the prevalence of some sort of *FMR1* gene mutation is surprisingly high, and all mutated allele forms can lead to clinical involvement of varying degrees.

The Phenotype of Fragile X Syndrome Consists of Physical, Behavioral, and Cognitive Abnormalities

Physical Characteristics Are Related to Connective Tissue Dysplasia and Endocrinological Irregularities

The classical physical phenotype of FXS includes large and prominent ears and forehead, a long narrow face, macrocephaly, and macroorchidism (large testicles). Females and young children tend to show subtler signs than males, and many do not show any obvious physical features at all. The physical phenotype found in FXS is thought to be related to a connective tissue dysplasia, and this dysplasia can also cause additional physical features including hyperextensible finger joints, double-jointed thumbs, a high-arched palate, flat feet, pectus excavatum, and soft, velvet-like skin (Fig. 1).

Many of these physical features in FXS, in particular the long face and macroorchidism, only begin to show up during puberty. (See Table 1 for pre- and postpubescent comparison.) Although puberty onset is usually normal in boys, there have been several reports of precocious puberty in girls with the full mutation. Throughout prepubertal childhood, individuals with FXS tend to demonstrate increased stature over controls, but their growth spurt is dampened in puberty, so their typically developing peers often are taller as adults. A limited number of endocrinological studies in FXS have implicated a defect in hypothalamic function in the absence of FMRP and dysregulation of growth hormone secretion, which accounts for the abnormal growth patterns. This may also relate to the acromegalic appearance in fragile X, such as a prominent jaw or long face in adulthood, but further study is needed to clarify this issue.

Fragile X Causes a Number of Medical Issues

A number of medical problems often occur in FXS, especially during the first few years of life, which are reviewed in Table 2. Many of these problems may be related, at least in part, to the connective tissue dysplasia, including joint dislocations, otitis media infections, hernias, and gastroesophageal reflux. Recurrent otitis media, which may be exacerbated by a collapsible Eustachian tube, is a problem for the majority of children with FXS. The angle of the Eustachian tube may be additionally altered by the long face and high-arched palate common in FXS. Both these factors adversely affect drainage, increasing the risk for infection. It is imperative to treat otitis media aggressively in children since it is associated with a conductive hearing loss, thereby further exacerbating the cognitive and language deficits already prevalent in FXS. This often requires the insertion of polyethylene (PE) tubes in the tympanic membranes or the use of prophylactic antibiotics for children with histories of recurrent otitis media.

Ophthalmological problems are common in FXS, including strabismus and nystagmus. Refractive errors, including myopia or hyperopia, can also occur, and many children with FXS need glasses at a young age. These ophthalmological conditions should be treated early on, particularly strabismus which may require

Fig. 1 A male with FXS exhibiting the typical physical features of an elongated face, prominent ears, prominent forehead, and a slight acromegalic appearance, particularly in the hands

Table 1 A list of physical features commonly found in FXS, pre-and postpubescence

	Prepubertal[a]		Postpubertal[a]	
	Male (%)	Female (%)	Male (%)	Female (%)
Physical feature	$n = 103$	$n = 40$	$n = 64$	$n = 27$
Long face	50	48	80	59
Prominent ears	69	68	66	30
High-arched palate	62	53	63	81
Hyperextensible finger joints	72	60	49	30
Double-jointed thumbs	55	38	48	30
Single palmar crease	22	15	22	11
Hand calluses	13	0	52	0
Flat feet	72	60	60	26
Heart murmur or click	1	0	29	19
Marcoorchidism[b]	39	n/a	92	n/a

Table adapted from Hagerman and Hagerman (2002), Tables 1.1 and 1.2
[a]Puberty cutoff defined as 13 years
[b]Macroorchidism defined as 3 mL or larger

Table 2 Medical concerns in males with FXS

Anxiety disorder (any)	86 % ($n = 58$)
Otitis media	85 % ($n = 291$)
Diagnosis of ADHD	80 % ($n = 224$)
Strabismus	36 % ($n = 161$)
Emesis	31 % ($n = 147$)
Diagnosis of autism	30 % ($n = 63$)
Diagnosis of PDD-NOS	30 % ($n = 63$)
History of sinusitis	23 % ($n = 43$)
Seizures	22 % ($n = 288$)
Glasses	22 % ($n = 148$)
Orthopedic intervention	21 % ($n = 171$)
Motor tics	19 % ($n = 188$)
Failure to thrive in infancy	15 % ($n = 138$)
Hernia	15 % ($n = 230$)
Psychotic ideation	12 % ($n = 146$)
History of apnea	10 % ($n = 139$)
Joint dislocation	3 % ($n = 150$)

Adapted from Hagerman and Hagerman (2002), Table 1.3
Updated with data from Harris et al. (2008) and Cordeiro et al. (2011)

surgery and/or patching before amblyopia (cortical loss of vision) sets in. An ophthalmological evaluation is recommended within the first 4 years of age and annually if problems are present. Normalizing vision and audition is essential for optimizing the development in FXS.

Hypotonia is also seen in the majority of young children with FXS after birth. When this problem is moderate or severe, the motor milestones are delayed, including sitting and walking independently. Such problems require physical and occupational therapies to improve both fine and gross motor coordination, truncal stability, and sensory integration deficits.

The connective tissue dysplasia can also cause mitral valve prolapse in adolescents and adults with FXS. Although it is usually benign, sometimes significant mitral regurgitation or arrhythmias occur. Sudden cardiac death is a rare occurrence in FXS but more frequent than in the general population. Another common issue is hypertension, though it is sometimes overlooked and dismissed as related to excessive anxiety in a medical clinic. When persistent, hypertension should be treated. Hypertension may be influenced by decreased resiliency of vessel walls secondary to abnormalities in elastin fibers and other connective tissue problems.

Another medical issue in FXS is sleep disturbances. Lowered FMRP expression has been linked to abnormal sleep-wake cycles in animal models of FXS, and wakefulness is common in children with FXS. These abnormal cycles can be a burden on the entire family. Not only do they cause stress and sleep deprivation in the patients and their caregivers, but sleep also plays an important role in learning and memory consolidation, especially during critical developmental periods in childhood. Furthermore, although these sleep problems, which can include night awakenings, bed-wetting, extended sleep latency, and trouble sleeping in unfamiliar

places, may diminish somewhat over time, they may not disappear completely. These problems can be treated, and pharmacological options have proven effective in this area, including clonidine and melatonin. Behavioral interventions, such as establishing a structured and consistent bedtime routine, can also be helpful.

Additionally, a subgroup of individuals with FXS display a phenotype of extreme obesity, delayed puberty, hypogenitalia, and hyperphagia called the Prader-Willi phenotype (PWP) because it is reminiscent of Prader-Willi syndrome, in which hypothalamic dysfunction is also present. The disruption of hypothalamic-mediated hormones in FXS can lead to the abnormal physical growth characteristics as well as to the increased insatiable appetite (hyperphagia) that drives the obesity. On a molecular level, FMRP interacts with a number of different proteins, among which is cytoplasmic interacting *FMR1* protein 1 (CYFIP1), which is found at chromosomal region 15q, a region that when deleted from the paternal side causes Prader-Willi syndrome. Reports have shown that lowered *CYFIP 1* expression levels are associated with the PWP.

Seizures are also a common problem, occurring in 20 % of individuals with FXS. Seizures associated with FXS also predispose to autism in this condition. The lack of FMRP in FXS causes an imbalance of excitatory (glutamate) and inhibitory (GABA) neurotransmitters, an imbalance which is thought to be the underlying pathophysiology of seizures in FXS. Seizures further interfere with CNS connectivity in FXS, which may underlie autism or ASD. Therefore, anticonvulsant medications, which suppress excess brain activity by altering neurotransmitter receptor permeability at the synapse, may be helpful in ameliorating both the social deficits as well as the severity of seizures in FXS.

The Behavioral Phenotype Is Exacerbated by Hyperarousal

The physical signs of FXS can be subtle and inconsistent, particularly among prepubertal children. Behavioral features, however, are more consistent across the spectrum of involvement in FXS. By age two or three, a child with FXS will typically present to a physician with speech delay and temperament difficulties. Tantrums, mood lability, and hyperactivity are often seen, as well as hyperarousal, sensory integration deficits, irritability, extreme anxiety, and autistic-like features such as perseveration in speech and behavior, gaze avoidance, and stereotypies such as hand flapping and hand biting. As the child ages, ID will become more and more pronounced as he falls further behind his typically developing peers. Difficulties with learning and attention regulation will become apparent as he begins school, as well as social deficits and sometimes aggression.

Many believe that the sensory integration and subsequent arousal modulation difficulties in FXS mediate many of the other behavioral problems. Physiological correlates of this observed hyperarousal include elevated heart rate, reduced vagal tone, increased sympathetic tone, increased cortisol levels, and increased electrodermal activity in response to challenging situations. Additionally, it takes an inordinately long time for these parameters to fall back to prestimulus levels. All this is indicative of a dysfunction of the autonomic nervous system. This imbalance causes their bodies to have greater reactivity or sympathetic response to environmental and

social stimuli. The behavioral features of fragile X, including social withdrawal or aggressive outbursts, may simply be a way of coping with the anxiety and sympathetic hyperarousal that occur with stimuli. Moreover, many of these behavioral issues are frequently observed during periods of transition, social interactions, and other high-demand, stimulating situations, indicative of an underlying hyperarousal trigger.

Attention Regulation and Hyperactivity Are Common Problems

Attention deficit/hyperactivity disorder (ADHD) is one of the most common comorbid diagnoses in FXS, even after controlling for the association between ID and ADHD. Moreover, many females and high functioning males with FXS without ID exhibit ADHD symptoms. Therefore, the *FMR1* gene appears to have a specific effect on attention, impulsivity, and hyperactivity.

ADHD is both a cognitive and a behavioral phenotype. The cognitive aspect (attention/focus) will be discussed in further detail below. The behavioral aspect consists of hyperactivity and impulsivity, which can often manifest as restlessness, fidgeting, excessive talking/moving, difficulty waiting, or blurting out answers before a question has been finished. Periods of transition can be especially difficult for many children with FXS, and they can be prone to impulsive outbursts of behavior when they do not get their way.

The hyperarousal and sensory integration difficulties in FXS may exacerbate the hyperactive/impulsive symptoms. Because these individuals have difficulty modulating their arousal levels from sensory stimuli, they have persistent, elevated sympathetic tone, predisposing them to hyperactive and impulsive behaviors. It is important for children with FXS to have physical outlets through which to dissipate their energy, such as being involved in sports or being the teacher's helper or messenger in school.

The downregulation of the inhibitory or GABA system and the upregulation of the glutamate system are associated with the impulsivity and hyperactivity that are part of the phenotype of FXS. The KO mouse model of FXS is also hyperactive, and treatment with a metabotropic glutamate receptor 5 (mGluR5) antagonist or a $GABA_A$ agonist will attenuate or even reverse the hyperactive phenotype in both the mouse and human with FXS.

Anxiety Afflicts Many Individuals with Fragile X

Anxiety is one of the hallmark behavioral signs of FXS, and shyness, or social anxiety, is often the presenting feature in females with FXS, many of whom may have intellectual abilities in the average range. Among males, it is often more difficult to assess anxiety because they may not be able to verbalize these symptoms. Nevertheless, anxiety is highly prevalent in both genders based on clinical observation and parent report and typically occurs with transitions, in social groups, or in new situations where sympathetic hyperarousal is manifested. Poor eye contact is usually seen in all patients with FXS, and this is associated with anxiety. Most

Table 3 Percentage of individuals with FXS meeting criteria for DSM-IV anxiety disorders based on the anxiety disorders interview schedule (ADIS-IV) clinical interview

Anxiety type	Total	Gender	
		Female	Male
Multiple disorders	58.3 %	55.3 %	60.3 %
Any disorder	82.5 %	76.9 %	86.2 %
Separation anxiety	11.5 %	18.4 %	6.9 %
Social phobia	36.5 %	39.5 %	34.5 %
Social phobia (adjusted)[a]	58.3 %	55.3 %	60.3 %
Specific phobia	59.6 %	51.4 %	64.9 %
Panic disorder	5.4 %	2.7 %	7.1 %
Agoraphobia	12.9 %	10.8 %	14.3 %
Generalized anxiety disorder	23.7 %	18.4 %	27.3 %
Obsessive-compulsive disorder	23.7 %	18.4 %	27.3 %
Posttraumatic stress disorder	4.3 %	5.4 %	3.5 %
Selective mutism	25.3 %	21.1 %	28.1 %

Table adapted from Cordeiro et al. (2011)
[a]Interview questions adjusted to be more appropriate for the FXS population. For details, see Cordeiro et al. (2011)

children with FXS meet DSM-IV diagnostic criteria for a variety of anxiety disorders, most notably social phobia, specific phobias, panic attacks, and selective mutism (see Table 3). Typically, these disorders persist into adulthood, and they are related to GABA and glutamate imbalances in the amygdala and insula, which are important brain structures for processing sensory stimuli and attributing emotional valence thereto.

There Is a High Comorbidity Between Autism and Fragile X

It is well established that autism and fragile X are interrelated. FXS is the leading single-gene cause of autism, and FMRP has been found to regulate the function of many genes that, when mutated, lead to autism, such as neuroligins, neurorexins, and SHANK proteins. In fact, FXS accounts for approximately 2–6 % of autism cases, and conversely, autism is also a very common comorbid diagnosis within FXS. Although precise prevalence estimates of some form of autism spectrum disorder (ASD) in FXS vary depending on the study and the instruments used, work at our center using the gold standard measures of ADOS (Autism Diagnostic Observation Schedule), ADI-R (Autism Diagnostic Interview – Revised), and DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders* – 4th edition) criteria shows approximately 30 % of individuals meet criteria for full autism, while an additional 30 % meet criteria for PDD-NOS, resulting in a total of 60 % who meet the criteria for some form of ASD. These numbers are in rough agreement with most other studies of this nature.

Diagnosing ASDs in fragile X can be a difficult task for two primary reasons. First, due to the molecular overlaps between FXS and autism, many autistic tendencies (such as gaze avoidance, stereotypies, and perseverative behaviors) are present

even in those who may never end up meeting diagnostic criteria for an ASD. Second, confounding behaviors common to the FX phenotype such as shyness, anxiety, and hyperarousal can negatively impact the quality of social interactions and skew the rating of a clinician at unpredictable times. Nevertheless, many studies have established that ASD is a common subphenotype within FXS that is associated with a lower IQ, more severe language deficits, greater impairments in adaptive behavior, and more frequent seizures than FXS without autism. Longitudinal evaluations have also suggested that this diagnosis is fairly stable over time, as in idiopathic autism.

Despite the association, there is nevertheless some evidence of qualitative differences between idiopathic autism and autism within FXS. In general, the autism symptomatology tends to be milder in FXS and tends to be mediated by hyperarousal and anxiety more than the social indifference or aloofness that is common in idiopathic autism. For example, an approach-withdrawal pattern of social interaction is common in FXS, wherein social motivation and interest are present despite an inability to maintain an appropriate interaction. The prototypical fragile X handshake, which consists of gaze aversion and a turning away of the body even while advancing toward a person and shaking hands, illustrates this concept well.

The presence of autism in FXS is also correlated with a higher rate of medical problems, particularly those related to CNS dysfunction, such as seizures, and additional genetic disorders like Down syndrome or the Prader-Willi phenotype of FXS.

It is important to recognize autism early in FXS because an intensive early intervention has been shown to be efficacious in autism, such as applied behavioral analysis (ABA) or the Early Start Denver Model, and should be started as early as possible to optimize development.

Intellectual Disability Affects Most Males and Some Females

There is a wide range of intellectual abilities in FXS. The majority of males and about 30 % of females meet the criteria for an intellectual disability (ID; IQ < 70). Additionally, IQ tends to decline throughout childhood and adolescence as fragile X children fall further and further behind their typically developing peers. Even in those females without ID, learning disabilities, ADHD, and emotional problems are common, as well as specific cognitive domains of weakness such as arithmetic and visual-spatial reasoning. In both genders, the severity of the cognitive limitations has been correlated with the magnitude of FMRP deficit, which explains why mosaics and females, particularly those with a high activation ratio, tend to have subtler cognitive deficits.

Numerous studies that have examined the patterns of strengths and weaknesses in FXS implicate executive function deficits even in those with a normal or near-normal IQ. Executive function is a top-down process mediated by frontal and prefrontal circuitry by which more basic cognitive processes are regulated to perform goal-directed behaviors. The dysfunction thereof adversely affects multiple domains

of cognition and behavior, such as attention, hyperactivity, impulsivity, and inhibitory processes. These problems are related to chemical imbalances in neurotransmission involving the cholinergic, dopaminergic, GABA-ergic, and glutamatergic systems.

Attention and working memory are two aspects of executive function that demonstrate pronounced deficiency in FXS. They are both related constructs that tap into overlapping circuitry within the parietal and frontal lobes and impact general information processing. This has clear repercussions in the academic domain but can also affect the social domain by creating difficulties in attending to and retaining pertinent social cues. More generally, attention/working memory deficits impact general reasoning ability by reducing the number of cognitive inputs that can be juggled in the head at any given time from which to extrapolate inferences for cognitive outputs. Furthermore, the nature of the attention deficit in FXS consists of a heavy emphasis on selective attention and inhibitory control. Behavioral manifestations of these deficits are broad but can be observed, for example, in echolalic or perseverative speech and repetitive motor patterns, which reflect an inability both in suppressing inappropriate prepotent lexical or motor neural signals as well as an inability to selectively boost appropriate ones.

Distinct Neurological and Neuroanatomical Differences Exist

Neurological findings in FXS include oculomotor disturbances (strabismus or nystagmus), ataxia, hypotonia, and hyperreflexia. Electroencephalogram (EEG) readings indicate a slowing of background brainwave activity, as well as abnormal intermittent theta and delta rhythms, with slower alpha waves. This is in contrast to the beta and alpha waves that should otherwise predominate in the waking mind in typically developing individuals. The significance of these abnormalities is not yet clear, but abnormal bursts of electrical activity, indicative of epilepsy, can also occur in 30–50 % of individuals with FXS, particularly in the centrotemporal region. However, the course of epilepsy in fragile X usually resolves after late childhood or adolescence, and typically, individuals respond well to anticonvulsive medications, which are important in preventing further brain dysfunction as a result of recurrent seizures.

The neuroanatomy of FXS has a number of unique features. Volumetric studies have shown general reductions in frontal and temporal lobes and enlargements in occipital and parietal lobes. Enlargements are also observed in certain specific structures such as the caudate nucleus, hippocampus, thalamus, and fusiform gyrus, even after controlling for overall brain volume. These areas are vital for various tasks, including memory, learning, information and sensory processing, facial processing, and social-emotional behavior, all of which are impaired in FXS. Additionally, there is a reduction in size relative to controls in the insula and cerebellar vermis. The former has been implicated in the manifestation of anxiety, as well as processing of sensory and emotional stimuli in general, and sensory integration, while the latter not only facilitates motor control but influences cognition and

behavior as well. Reduction in vermal volumes, as well as increases in caudate nucleus volume, both correlate with some autistic symptoms and cognitive performance in individuals with fragile X.

The volumetric changes are singular to the *FMR1* gene mutation and are not found in groups of idiopathic developmental or intellectual delay. They are also present in very young toddlers, indicating that the abnormal brain development may take place prenatally, mediated by factors related to the *FMR1* gene mutation and not by exogenous environmental factors. In fact, many of these structural changes in the brain correlate with blood levels of FMRP.

Compared to typically developing children and those with idiopathic autism, young children with FXS have a smaller amygdala and a larger caudate, whereas those with idiopathic autism have a larger amygdala. Although the size of the amygdala in autism decreases with age, it remains larger than what is seen in FXS. Functional MRI (fMRI) studies also show deficits of activation in many areas of the CNS, as well as activation of aberrant neural pathways, compared to age-matched controls, particularly when solving complex problems. The deficits of fMRI activation correlate closely with deficits of FMRP.

The Phenotype of Premutation Carriers Is Related to RNA Toxicity

Premutation carriers of the *FMR1* gene (between 55 and 200 CGG repeats) were once thought to be clinically unaffected, whose only risk was to have the gene expand into a full mutation in successive generations. However, evidence in the past decade or so has been slowly accumulating, pointing to distinct clinical manifestations in premutation carriers. The two most overt pathologies are FXPOI and FXTAS, each described in further detail below. However, a range of clinical issues beyond these two disorders may be present in some premutation carriers, including psychiatric problems, fibromyalgia, hypothyroidism, hypertension, neuropathy, migraine headaches, and irritable bowel syndrome.

Fragile X-Associated Primary Ovarian Insufficiency Affects Women with the Premutation

Approximately 20 % of female carriers experience cessation of menstrual periods prior to age 40 and sometimes as early as their teenage years or twenties. Even those who do not experience such overt symptoms as cessation of menses tend to show elevated levels of follicle-stimulating hormone as well as other signs of diminished ovarian function such as decreased levels of anti-mullerian hormone, inhibin A, and inhibin B or irregularities in menstrual cycles. This condition was initially termed premature ovarian failure (POF) but has been subsequently renamed to fragile X-associated primary ovarian insufficiency (FXPOI) because an occasional carrier can become pregnant after cessation of periods. This condition afflicts approximately 20 % of all female premutation carriers, which is significantly different

from the 1 % of women in the general population who have POI. For reasons that are still unclear, there is a nonlinear correlation between CGG repeat size and penetrance of FXPOI, such that penetrance increases with the CGG repeat size up to approximately 100 repeats, after which penetrance declines, though still remains elevated above the normal population. One possible explanation for this nonlinear penetrance is the higher propensity for large-repeat (>100) carriers to have a proportion of their oocytes carrying the full mutation allele, which is protective against FXPOI and the RNA toxicity hypothesized to underlie much of the premutation involvement.

Fragile X-Associated Tremor/Ataxia Syndrome Affects Most Aging Men with the Premutation and Some Women

FXTAS is a neurodegenerative disorder that typically affects many male premutation carriers in their early 60s. For some, onset can begin as early as the 50s or late 40s, and females can also be affected, though more infrequently and less severely.

Principal symptoms include intention tremor, cerebellar ataxia, parkinsonism, autonomic dysfunction, cognitive decline, and peripheral neuropathy. Tremor and ataxia, in that order, will typically be the harbingers that usher in the rest of the syndrome. Signs of peripheral neuropathy occur in many, including numbness and tingling in the extremities, as well as decreased reflexes and vibration sense. From there, ambulation and general motor ability will progressively decline in males and less frequently in females. Dysautonomia is also common, involving impotence, blood pressure dysregulation, urgency, and incontinence. Psychiatric symptoms are common prior to the development of FXTAS, but with the onset of motor problems, symptoms such as anxiety, mood instability, agitation, apathy, and depression become worse. As neurodegeneration progresses in the brain, cognitive changes will also become apparent, such as executive function deficits including disinhibition, memory loss, and, in some cases, eventual dementia. However, mounting evidence suggests that some of these psychiatric and cognitive aberrations, particularly the executive dysfunction, may be subtly present in some premutation carriers at a younger age. The prevalence of FXTAS increases with age, and by the 80s more than 75 % of premutation males will develop FXTAS. The prevalence of FXTAS is lower in females with the premutation, and up to 17 % will experience this disorder. FXTAS tends to run in families, so the risk of FXTAS is increased if others in the family have this disorder. RNA toxicity is thought to be the cause of premutation problems at any age.

Radiological findings in FXTAS include cerebral, brainstem, and cerebellar atrophy, as well as increased T2 signal intensity in the white matter of the middle cerebellar peduncles (MCP sign), the insula, the periventricular area, and the subcortical region and deep white matter of the cerebellum and cortex. The cause of the white matter disease is related to axonal disease and/or neuronal cell death secondary to RNA toxicity. On rare occasions, the MCP sign can be seen before the onset of FXTAS symptoms of tremor and ataxia. The radiological findings correlate with the severity of cognitive changes and the overall clinical stage of FXTAS, which is rated

on a scale of 0–6 of increasing symptomatology. Females with FXTAS have less severe changes on MRI compared to males with FXTAS, and sometimes symptoms of tremor and ataxia are associated with a normal MRI in females. Table 4 details the clinical and radiological signs of FXTAS, as well as the various stages of involvement.

Neuropathological findings associated with FXTAS include the presence of eosinophilic intranuclear inclusions in neurons and astrocytes throughout the brain. These inclusions are unique to FXTAS and are tau and synuclean negative, but they contain the elevated *FMR1* mRNA, and they also sequester many other proteins important for cell function including Sam 68 and DROSHA, which is an important protein for processing micro RNAs (miRNAs). Therefore, miRNAs are dysregulated in individuals with FXTAS, and this is likely part of the toxicity of elevated mRNA.

Recent studies of mitochondrial function in fibroblasts and muscle cells in premutation carriers have demonstrated abnormalities of mitochondrial function that increase in severity as FXTAS becomes more advanced. This finding is consistent with the progressive weakness and sedation that most individuals with FXTAS experience. All complexes of the mitochondria appear to be involved, and

Table 4 Diagnostic criteria, categories, and stages for FXTAS

	Clinical signs	Radiological signs
Major	1. Intention tremor	1. MRI white matter lesions involving middle cerebellar peduncles
	2. Cerebellar gait ataxia	
Minor	1. Parkinsonism	1. MRI lesions involving cerebral white matter
	2. Moderate to severe working memory deficit	2. Moderate to severe generalized brain atrophy
	3. Executive function deficit	
Diagnostic categories		
Definite	One major clinical and one major radiological sign	Presence of FXTAS inclusions
Probable	Two major clinical signs	One minor clinical and one major radiological sign
Possible	One major clinical and one minor radiological sign	
FXTAS stages		
0	Normal function	
1	Subtle or questionable signs, that is, subtle tremor or mild balance problems, but no interference with activities	
2	Minor, but clear, tremor and/or balance problems: minor interference with activities	
3	Moderate balance and/or tremor problems interfering with ADLs and at least occasional falls	
4	Severe tremor and/or balance problem. Requires assistance with ADLs and uses a cane or walker	
5	Uses wheelchair on a daily basis	
6	Bedridden and cannot perform ADLs (activities of daily living)	

Adapted from Berry-Kravis et al. (2007)

there is a problem in transport of at least three nuclear encoded enzymes into the mitochondria needed for normal function. This mitochondrial dysfunction begins before the onset of FXTAS. Many individuals with FXTAS remember their tremor or ataxia beginning initially after a prolonged surgery with general anesthesia. It is possible that an environmental toxin, such as anesthesia, can precipitate FXTAS. Studies of neuronal cell cultures of premutation neurons demonstrate enhanced cell death by 21 days in culture compared to control neurons without the premutation, suggesting a vulnerability of the neuron to early cell death because of RNA toxicity.

RNA Toxicity Can Cause Psychopathology and Behavioral Abnormalities in Premutation Carriers

Aside from FXPOI and FXTAS, a number of other clinical concerns affect premutation carriers. Approximately 50 % of carriers experience significant anxiety or depression at some point in their lives, including childhood. Poor eye contact can also be a problem for some children and adults with the premutation. Social anxiety is commonly seen, and this can lead to social avoidance in some carriers. About 10 % of boys with the premutation will meet diagnostic criteria for autism or ASD in childhood, and this rate is increased if the patient presents clinically. Approximately 35–50 % of male carriers will meet criteria for ADHD. These problems usually occur without intellectual disability, and they are thought to be caused by a developmental effect of RNA toxicity. Seizures occur in about 13 % of carriers, and if they occur in childhood, they increase the risk of ASD. Perhaps the seizures further interfere with brain connectivity in premutation carriers, or perhaps they increase cell death, but they should be vigorously treated in childhood. Anxiety symptoms usually respond well to an SSRI such as sertraline or fluoxetine, and ADHD symptoms respond well to stimulants.

Neuronal cell cultures of premutation neurons demonstrate that the dendritic spines have decreased length and complexity and slower movement of the mitochondria in the axons and dendrites compared to wild-type neurons. These various irregularities may contribute to the finding of mild cognitive problems, social deficits, and behavioral issues in some carriers. Although there seems to be no impairment in gross cognitive functioning, and overall IQ in carriers tends to be in the average range, a number of studies have revealed subtle deficits in specific domains, such as executive function, which can start off manifesting as inattention and progress to disinhibition with age. The neuropsychological profile of premutation carriers is still being explored, and further studies are needed to understand why some carriers have deficits and most others do not.

Psychiatric problems including mood and anxiety disorders are also common in adulthood in carriers. Major depressive disorder is reported in many carriers, even in males who typically show lower rates of depression in the general population, as well as social phobia, generalized anxiety disorder, and obsessive-compulsive disorder (OCD). These issues are often reported to be present prior to the birth of fragile

X children in females and prior to the onset of FXTAS in males. Therefore, this psychiatric vulnerability appears to be related to intrinsic factors such as the RNA toxicity seen in carriers, independent of environmental stressors, although it can certainly be exacerbated by the latter.

Moreover, the premutation mouse model demonstrates an enhanced release of cortisone, the animal counterpart to the human stress hormone cortisol, with stress, and this is likely to also be the case in patients with the premutation. Perhaps the anxiety and OCD, as well as hypertension, that are prevalent in carriers are manifestations of this enhanced stress response. Relaxation techniques and yoga are likely to be helpful interventions. In addition, oxidative stress at the cellular level is common because of mitochondrial problems, so antioxidants are routinely recommended for those with a premutation or a full mutation.

A smaller percentage of carriers, particularly those with dramatically elevated levels of mRNA, may display psychotic features such as hallucinations and delusions, which may be part of a mood disorder or a primary psychotic process. This is more common when the premutation is associated with a full mutation as in a mosaic individual, although it occurs in less than 10 % of cases. Such symptoms require the use of an atypical antipsychotic agent such as aripiprazole or risperidone.

Targeted Treatments Are Being Developed for Fragile X

We have entered into a new age of targeted treatments in FXS that can reverse the neurobiological abnormalities associated with the loss of FMRP. In the absence of FMRP, which acts as a translational repressor, the metabotropic glutamate receptor 5 (mGluR5) system is upregulated, and the use of an mGluR5 antagonist has been shown to reverse many of the features of FXS in the mouse model, including seizures, hyperactivity, and cognitive deficits, in addition to the dendritic spine abnormalities of long, thin, and immature spines. Human trials of mGlur5 antagonists have been initiated, and preliminary studies are positive regarding behavior changes, although improvements in cognitive deficits have not yet been demonstrated. The gamma-aminobutyric acid (GABA) system is also downregulated in FXS, and the use of GABA agonists has also proven helpful in the knockout (KO) mouse model of FXS. Arbaclofen, a $GABA_B$ agonist that lowers glutamate at the synapse, has been shown to be helpful in children and adults with FXS that have ASD or significant social deficits. The $GABA_A$ agonist, ganaxolone, has also been tried in the KO mouse, with positive effects, and human trials are now being initiated.

Minocycline is also a targeted treatment for FXS because it lowers matrix metalloproteinase 9 (MMP9), which is remarkably elevated in FXS. MMP9, in high levels, alters dendritic spine morphology, creating long, thin, and tortuous spines. Minocycline has been shown to reverse this abnormality and help maturation of dendritic spines. Animal studies of newborn KO mice demonstrated improvements in synaptic maturity and behavior. Initial human trials in children and adults

with FXS have demonstrated benefits in behavior in a survey and in an open trial. Controlled trials are now taking place.

Although the use of targeted treatments suggest a bright future for FXS, the need for combined treatments with standard interventions, such as stimulants and SSRIs, should not be ignored. The need for innovative educational interventions such as applications in iPad technology, learning games, approaches to enhance attention and concentration such as CogMed, or other educational interventions also require more research, particularly in combination with targeted treatments that can strengthen synaptic connections.

Outlook

This is an exciting time in the treatment of neurodevelopmental disorders, and FXS is leading the way for targeted treatments for many other disorders. There is now evidence that several other disorders may have deficits of FMRP in the CNS, including schizophrenia and autism. It is likely that the targeted treatments for FXS will be helpful for many other disorders, and studies have already been initiated regarding the benefit of arbaclofen and minocycline in autism. Treatment for premutation disorders is just beginning, and medications that will reverse the neurotoxicity of elevated *FMR1* mRNA levels are progressing. The extensive manifestations of premutation involvement require more research, particularly the autoimmune problems that many females experience. For the clinicians interested in fragile X-associated disorders, there is much to learn, and assessment of the whole family tree is needed to provide optimal treatment and genetic counseling for the many individuals involved with these fascinating disorders.

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References

Berry-Kravis E, Abrams L, Coffey S et al (2007) Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Mov Disord 22(14):2018–2030

Cordeiro L, Ballinger E, Hagerman R et al (2011) Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. J Neurodev Disord 3(1):57–67

FRAXA Research Foundation. Retrieved February 8, 2011 from http://www.fraxa.org/. Accessed 5 Apr 2012

Hagerman R, Hagerman PJ (eds) (2002) Fragile X syndrome: diagnosis, treatment, and research, 3rd edn. The Johns Hopkins University Press, Baltimore

Hagerman R, Hall D, Coffey S et al (2008) Treatment of fragile X-associated tremor ataxia syndrome (FXTAS) and related neurological problems. Clin Interv Aging 3(2):251–262

Hagerman R, Berry-Kravis E, Kaufmann W et al (2009) Advances in the treatment of fragile X syndrome. Pediatrics 123(1):378–390

Harris S, Hessl D, Goodlin-Jones B et al (2008) Autism profiles of males with fragile X syndrome. Am J Ment Retard 113(6):427–438
National Fragile X Foundation. http://www.fragilex.org. Accessed 5 Apr 2012
Schneider A, Hagerman R, Hessl D (2009) Fragile X syndrome-from genes to cognition. Dev Disabil Res Rev 15(4):333–342
Wang L, Berry-Kravis E, Hagerman R (2010) Fragile X: leading the way for targeted treatments in autism. Neurotherapeutics 7(3):264–274