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Advances in clinical and molecular understanding of the *FMR1* premutation and fragile X-associated tremor/ataxia syndrome

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Summary

Fragile X syndrome, the leading heritable form of cognitive impairment, is caused by epigenetic silencing of the fragile X (*FMR1*) gene consequent to large expansions (>200 repeats) of a non-coding CGG-repeat element. Smaller, "premutation" expansions (55–200 repeats) can give rise to a family of neurodevelopmental (ADHD, autism spectrum disorder, seizure disorder) and neurodegenerative (FXTAS) clinical phenotypes through an entirely distinct molecular mechanism involving increased *FMR1* mRNA production and toxicity. Basic cellular, animal, and human studies have helped to elucidate the underlying RNA toxicity mechanism, while clinical research is providing a more nuanced picture of the spectrum of clinical involvement. Whereas advances on both mechanistic and clinical fronts are driving new approaches to targeted treatment, two important issues/needs are emerging: to define the extent to which the mechanisms contributing to FXTAS also contribute to other neurodegenerative and medical disorders, and to redefine FXTAS in light of its differing presentations and associated features.

Introduction

Many neurologists are not aware of the stark distinction between the neurodevelopmental disorder, fragile X syndrome (FXS), and the neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). They tend to conflate these mechanistically and clinically distinct entities because both arise from mutated forms of the fragile X mental retardation 1 (*FMR1*) gene. Although both disorders are caused by expansions of a trinucleotide (CGG) repeat element in a non-coding portion of the gene, FXS is caused by expansions to greater than 200 CGG repeats, whereupon the gene undergoes methylation-coupled silencing. The consequent lack of the *FMR1* protein (FMRP) – important for proper synapse development and function – causes intellectual disability and an autism spectrum disorder (ASD) in most males; and similar, albeit less severe intellectual and behavioral problems in females.

By contrast, FXTAS is clinically manifest almost exclusively among carriers of premutation repeat expansions (55 to 200 CGG repeats), where the *FMR1* gene remains fully active; indeed, the gene generally expresses elevated levels of its mRNA,[1] which is thought to result in CGG-repeat–induced toxicity of the mRNA and the adult-onset neurodegenerative disorder.[2–5] Thus, although FXS and FXTAS are caused by CGG-repeat expansions within

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the same gene, they affect different groups of individuals (i.e., possessing full mutation versus premutation alleles), are clinically apparent at opposite ends of the age spectrum, and arise through entirely different pathogenic mechanisms (gene silencing and RNA toxicity, respectively). The current review will focus on advances in our understanding of clinical involvement among carriers of premutation alleles, which include but are not limited to those with FXTAS.

Premutation Involvement

In the years before the discovery of the *FMR1* gene in 1991, and for some years thereafter, carriers of premutation alleles were considered to be clinically unaffected; their importance as a group being that female carriers of premutation alleles often had children with the genetic disorder, FXS. With the discovery of the gene, the basis of this propensity was recognized as being due to instability of the CGG-repeat, with dramatic premutation-to-full mutation expansions occurring during matrilineal transmission. However, shortly after discovery of the gene, Cronister et al.[6] reported that there was a much higher incidence (~20%) of early ovarian failure (before age 40) in female carriers relative to the general population (~1%). Fragile X-associated primary ovarian insufficiency (FXPOI) is now known to be the leading cause of early menopause in the general population.[7] Additional forms of involvement among carriers of premutation alleles were identified throughout the 1990s. Psychiatric problems, including depression and anxiety, gained recognition during this period,[8] though these clinical features were initially considered to be related to the stress of raising children with FXS. More extensive controlled studies utilizing validated psychiatric clinical interviews, such as the Structured Clinical Interview for DSM-IV Disorders (SCID),[9] have established that depression and/or anxiety occur in approximately 40% of carriers.[10–14] We now know that carriers are intrinsically more vulnerable to depression and anxiety, because these problems can occur prior to having children affected by FXS.[10, 14] However, this intrinsic vulnerability is also sensitive to the stress of raising a child with FXS and/or caring for a parent with FXTAS.[15–17]

The discovery of FXTAS in 2001[3, 4] brought a new, more disturbing dimension to premutation involvement. FXTAS is a progressive neurodegenerative disorder involving core features of intention tremor and gait ataxia, with associated white matter disease and global brain atrophy.[2, 4, 5, 18–21] The identification of FXTAS helped to validate earlier reports of nebulous emotional or neuropsychiatric involvement in premutation carriers, essentially by anchoring the severe end of a spectrum of clinical involvement experienced by carriers of premutation alleles.

Prevalence of the Premutation

The premutation allele is relatively common in the general population, with estimates of prevalence dependent upon the population being assessed. In Israel, approximately 1 in 113–157 females is a carrier.[22] In Spain, estimates of carrier prevalence (1 in 130 females and 1 in 250 males; n=5,235; CGG repeat: range, 10–77; modal value, 30) utilized newborn bloodspots collected previously as part of the national newborn screening program, which were provided to the investigators as consecutive, de-identified samples, thus largely avoiding sample bias.[23] In a Wisconsin study, the corresponding numbers were 1 in 151 females and 1 in 468 males (n=6,747; CGG repeat: range, 9 to 135; modal value, 31);[24] this population-based sample (saliva-derived DNA) was restricted to high-school graduates, thus introducing a possible bias toward reduced prevalence. In a more recent bloodspot screening study in California, 1 in 209 females and 1 in 400 males were found to be carriers (n=14,207; CGG repeat: range, 6 to 44; modal value, 30).[25] This last study was subject mainly to a bias associated with consent procedures for newborn screening, although a

subset of the sample was tested anonymously after de-identification of the newborn blood spot.[25]

Neurodevelopmental Problems Associated with the Premutation

Accompanying the growing awareness of clinical involvement in adult carriers of premutation alleles is an increasing appreciation of neurodevelopmental problems, which are more common among boys with the premutation who may suffer from a higher rate of attention deficit hyperactivity disorder (ADHD), shyness, social deficits, autism spectrum disorders (ASD), and (occasionally) intellectual disability.[26–29] The prevalence of developmental/behavioral problems, including ASD or ADHD, is higher in boys who present clinically with a behavioral problem than in other family members identified with cascade testing (ASD 8%; ADHD 30%).[26, 27, 30]

It is not known at present why some carriers, but not others, will experience some form of neurodevelopmental involvement; nor is it possible to predict, based on the presence or absence of neurodevelopmental features, whether an individual will eventually develop FXTAS. Although the severity of amygdala dysfunction in young adult males[12] and the age of onset of FXTAS[31, 32] both correlate with the premutation CGG-repeat expansion, penetrance remains incomplete. This observation suggests that additional genetic, epigenetic, and environmental factors influence disease formation. Premutation carriers with larger CGG repeats are more likely to have mild deficits in FMRP, developmental problems,[28] and manifest altered amygdala function.[12] Deficits in FMRP lead to up-regulation of the mGluR5 system[33] and down-regulation of the $GABA_A$ system,[34] resulting in an excitatory/inhibitory imbalance that is observed in the premutation mouse-model neurons.[35] In this regard, a recent transcranial magnetic stimulation (TMS) study[36] demonstrated that asymptomatic adult women with the premutation have reduced $GABA_A$-mediated intracortical and afferent inhibition from the cerebellum to the primary motor cortex, compared to age-matched controls. It is possible that these $GABA_A$ deficits predispose carriers to the ADHD symptoms experienced by approximately 30% of boys with the premutation.[26, 27] The $GABA_A$ deficits can also lead to a higher frequency of burst-type firing in networks of cultured premutation mouse neurons,[35] which likely represents the basis for the increased rate of seizures (8 to 13%) in carriers.[27, 37, 38] Moreover, the presence of seizures is significantly associated with the development of ASD in boys with the premutation.[27] The association between seizures and ASD is also found in other neurodevelopmental disorders including tuberous sclerosis and neurofibromatosis.[39] These data suggest that early treatment of seizures is important in premutation carriers and in other disorders associated with autism or ASD.

Clinical and Radiological Features of FXTAS

The original descriptions of FXTAS emphasize a presentation of intention tremor and/or gait ataxia with an onset typically between 60–65 years of age.[3, 4, 40] Intention tremor and ataxia are generally considered the core features of FXTAS and are currently major diagnostic criteria for FXTAS (table 1).[4, 41] Diagnostic criteria also include memory problems and EF deficits. Additionally, Brunberg et al.[18] recognized that the middle cerebellar peduncle (MCP) was a distinctive feature of the white matter disease detected by T2 FLAIR MR imaging in those with FXTAS. Because this MCP finding was highly specific (albeit not unique) to FXTAS, it was designated as a major criterion for the diagnosis of definite FXTAS. The MCP sign is found in approximately 60% of men,[5, 19] and those with FXTAS and the MCP sign are likely to have more severe cognitive deficits and a longer history of symptoms than those without the MCP sign.[5] The MCP sign is seen in only 13% of women with FXTAS.[19] Additional common neuroimaging signs of FXTAS include white matter

hyperintensities in the pons, insula, splenium of the corpus callosum (CC), and periventricular region.[2, 21, 42, 43] In addition, thinning of the CC,[2] cerebellar atrophy,[44] and white matter abnormalities, as assessed by diffusion tensor imaging (DTI), particularly involving frontocerebellar tracks, can be seen in those with FXTAS and in adult asymptomatic premutation carriers who do not have FXTAS.[45–47] These findings suggest that the RNA toxicity initiates brain changes well before the onset of FXTAS.

Although neuropathy was recognized early as a common clinical problem in those with FXTAS,[4] it was not included in the initial diagnostic criteria. Subsequently, neuropathy was established as a common early symptom in carriers who later developed FXTAS,[48–50] and was observed in 81% (18/22) of patients with FXTAS.[2]

Poston and co-workers[51] noted a generalized reflex myoclonus in a 60-year old patient with FXTAS, and showed that tapping deep tendons in the arms or legs with a reflex hammer resulted in single jerks of the neck, trunk, abdomen, and arms. This same reflex myoclonus occurred when stroking the palm or soles with a key.[51] In our experience, this finding is common with advances cases of FXTAS, particularly with frontal release signs such as a snout reflex.

Parkinsonism is a minor diagnostic criterion of FXTAS[4] and has been subsequently documented in 67% of 22 individuals with FXTAS.[2] Single photon emission computerized tomography (SPECT) studies of the nigrostriatal system, specifically the dopamine transporter density (DAT), have shown deficits with significantly reduced uptake of [^{123}I] FP-CIT in the putamen bilaterally in patients with FXTAS.[52–54] These findings demonstrate not only cerebellar problems, but also basal ganglia dysfunction in FXTAS, though dopaminergic dysfunction is not always present in those with FXTAS.[55]

Typically, the age of onset of FXTAS is in the early 60s, and presents with onset of tremor.[40] However, DTI findings suggest that there are asymptomatic changes in the brain associated with the pathologic changes of FXTAS well before the onset of tremor or ataxia.[45–47] Typical early symptoms include neuropathy and erectile dysfunction, or other autonomic symptoms such as episodes of hypotension; however, occasionally cognitive decline is the initial symptom.[56] Juncos and colleagues[57] found that an essential tremor can last for years or sometimes decades before the onset of ataxia, which then marks a stage of more rapid decline. Subsequently falls begin 6 years, use of a cane 15 years, and death 21 years after initial symptoms, on average.[40] In the Leehey et al. study,[40] life span post-diagnosis of FXTAS ranged from 5 to 25 years.

Recent studies have documented a number of new symptoms associated with FXTAS, as outlined in table 2. Olfactory dysfunction has not been previously reported, but was found in 80% of patients with definite FXTAS.[57] As individuals progress from the possible through definite stages of FXTAS, these symptoms in table 2 become more common. In late stage FXTAS, for instance, problems with swallowing, urinary and bladder incontinence, sedation in the day, and muscle weakness are more common.[40, 57, 72, 73]

Neuropsychological Deficits

Some FXTAS-associated cognitive problems, including memory and/or executive function deficits, are often present at the time an individual exhibits tremor and ataxia;[5, 74–77] however, many men with FXTAS have a history of attention problems in childhood that may have led to a diagnosis of ADHD.[26, 27, 78] Women with the premutation also have an increased rate of ADHD prior to the onset of FXTAS.[79] Sevin et al.[52] surveyed all known carriers over 50 years of age in France and identified many with cognitive decline without obvious motor deficits. Grigsby et al.[75] also reported executive function deficits in a

subgroup of older carriers who did not have tremor or ataxia. Hunter et al.[80] reported that the presence of executive function deficits is not a significant finding in carriers who are under age 50. However, their conclusion stands in contrast to other reports[76, 78, 81] where such deficits were found to be increased in premutation carriers without FXTAS, including some under age 50. The difference in these findings is related to different measures used to detect executive function deficits,—the Behavior Dyscontrol Scale is very sensitive to these deficits in carriers and the Hunter study did not use this measure.[74–76] Yang et al.[77] showed that the executive function deficits in FXTAS patients correlated with Event Related Potential (ERP) changes, particularly in P3 with reduced amplitude and latency on an auditory "oddball" paradigm. The P3 component of the ERP is known to relate to selective attention, attention orienting to salient or unexpected events, and to working memory. Smaller P3 amplitudes correlated with higher CGG repeats and higher *FMR1* mRNA levels in those with FXTAS. The executive function and visual spatial processing deficits deteriorated over time, leading to a broader cognitive decline, although Brega et al.[76, 82] have documented that the executive function deficits mediate the other secondary cognitive changes in learning, memory, and visual spatial processing.

Neuroimaging studies have corroborated the ERP findings with volumetric studies, demonstrating significant atrophy in several frontal brain regions, including the dorsomedial and dorsolateral prefrontal cortex, the orbitofrontal and anterior cingulate cortex, and the medial parietal (precuneus) and superior parietal cortex.[44] Executive function deficits are also documented in a functional MRI study[83] of a verbal working memory task in carriers. The authors found reduced activation in core working memory areas, right ventral inferior frontal cortex, and left premotor/dorsal inferior frontal cortex for both premutation carriers with and without FXTAS, compared to healthy controls.

Seritan et al.[84] have shown that 42% with late stage FXTAS develop significant cognitive decline and meet diagnostic criteria of dementia. Dementia is far less common in women,[84] though in a recent neuropathology study of eight women with the premutation, four were diagnosed with dementia prior to death, and almost all had some neuropathological features of Alzheimer's disease at autopsy.[85] All eight cases possessed intranuclear inclusions, although the diagnosis of FXTAS was not recognized before death in approximately half of the cases.

Pathogenesis of Premutation Disorders and FXTAS

Triggering events

Evidence accumulated to date strongly favors a triggering event involving sequestration of one or more proteins by the expanded CGG repeat in the *FMR1* mRNA 5′UTR (figure 1a) (recent reviews:[86, 87, 88]). By analogy with protein sequestration in myotonic dystrophy (recent reviews:[89, 90]), the RNA "toxicity" in FXTAS is thought to involve the sequestration of one or more RNA binding proteins, thus reducing their capacity to carry out their normal functions. Candidate proteins include hnRNP A2/B1,[91, 92] Pur α,[93, 94] Sam68,[95] and DGCR8.[96, 97] For all of these proteins, there is evidence for *in vitro* association with CGG-repeat-containing RNA, as well as *in vivo* evidence of a functional role in mediating effects of the CGG-repeat RNA. However, for none of these candidates, except perhaps DGCR8, is there a compelling functional linkage to FXTAS neurodegeneration, underscoring the need for continued research. Although there exists strong evidence for the role of Pur α in mediating neurodegeneration in *Drosophila*, it has not been detected in the intranuclear inclusions in murine models (reviewed in:[98]), and its presence in human inclusions remains inconclusive.[91, 98] In contrast to the situation with Pur α, there is growing evidence for a role of hnRNP A2/B1 in mediating at least some aspects of neurodegeneration, not only in *Drosophila*,[92, 99, 100] but also in cultured rat neurons,[101] where the RNA binding protein is

thought to facilitate mRNA dendritic transport. Although a number of candidates as protein mediators of the CGG-repeat mRNA toxicity exist, more work needs to be done in mammalian systems to clarify their respective roles, if any, in the pathogenesis of premutation-associated disorders, including FXTAS. Finally, it should be noted that while the preponderance of evidence favors the CGG-repeat–mediated, protein-sequestration model, other models for RNA toxicity have been proposed. These include the possibility that the CGG-repeat–containing RNA initiates a conformational transition in one or more proteins that possess prion-like domains,[86] analogous to amyloid plaque formation in Alzheimer's disease; or that non-ATG (RAN) translation is occurring, yielding altered polypeptide products that could themselves lead to aggregation and neuronal toxicity.[102–104] Such a mechanism has been proposed for expanded hexanucleotide (G_4C_2)-repeat alleles of the C90RF72 gene, a genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis (FTD/ALS). Recently, Todd et al.[104] have demonstrated the presence of RAN translation products from the *FMR1* mRNA involving initiation of protein synthesis upstream of the CGG repeat and a shift in codon frame (CGG-to-GGC). The resulting peptide includes a polyglycine-containing peptide and additional downstream peptide sequence. The relative importance of this mechanism to FXTAS neurodegeneration awaits further study. However, it is interesting to note that a premutation mouse model[105] that presumably does not support RAN translation[104] displays significant Purkinje cell dropout, as is the case with FXTAS in humans.

Features of cellular dysregulation

Although there are myriad possibilities for downstream domains of cellular dysregulation – processes that actually make the cells sick – suggested by the above-mentioned protein candidates, two general processes are beginning to emerge as core mediators of cellular dysregulation and dysfunction; specifically, altered mitochondrial function[106–108] and altered calcium (Ca^{2+}) regulation[35] (figure 1b). Evidence for mitochondrial dysfunction exists for both cultured fibroblasts from adult premutation individuals with and without FXTAS, and from *postmortem* brain tissue from FXTAS patients. Several general features are apparent,[106] including lower oxidative phosphorylation capacity, lower mitochondrial levels, and higher precursor-to-mature ratios of several nuclear-encoded subunits of electron transport complexes, indicative of a defect in mitochondrial protein import. This mitochondrial functional deficiency may explain the evidence of increased oxidative stress in CNS tissue.[107] In this regard, Qurashi et al.,[109] observed an accumulation of transcripts from stress response genes in the *Drosophila* model of the premutation, suggesting a cellular response to oxidative stress, and/or to mitigate an inflammatory response; such inflammatory or immune-mediated processes do co-occur in those with FXTAS at a higher rate than expected in the general population.[49, 68, 70, 85, 110, 111]

More recently, Kaplan et al.[108] demonstrated that hippocampal neurons from premutation knock-in mice (~170 CGG repeats) have fewer mitochondria, with lower mitochondrial mobility and higher rates of basal oxygen consumption and proton leak. Importantly, these hippocampal neurons were obtained from neonatal mice, which do not manifest any overt features of neurodegeneration. Mitochondrial dysfunction could be a very early feature of the incipient processes that ***may*** eventually lead to FXTAS in adulthood.

Also evident in the neonatal period are abnormalities of neuronal morphology and network activity (mouse hippocampal neurons), as well as altered Ca^{2+} regulation.[35, 112] Remarkably, the burst-type firing observed with the premutation mouse neurons on multi-electrode arrays, perhaps related to the heightened seizure activity in childhood for premutation carriers, is completely reversed with mGluR5 antagonists, or the $GABA_A$ receptor positive modulator, allopregnanolone.[35] These observations suggest that there are

overlapping downstream pathways between premutation and full mutation disorders, even if the initial triggering events (RNA toxicity; FMRP deficiency) are distinct.

Additional factors leading to neurodegeneration and FXTAS

One of the puzzling aspects of the neurodegenerative process leading to FXTAS is why it only affects some individuals with a given CGG-repeat size, whereas others appear to be spared. This issue is of particular importance, since essentially all mice that carry comparably sized CGG repeats do demonstrate early developmental neuronal cell dysfunction, and later inclusion formation, but they do not suffer the debilitating neurodegenerative process experienced by a portion of humans who are premutation carriers. Part of the answer to this apparent paradox is likely to involve additional genetic and/or environmental factors, such as smoking,[7] environmental toxins,[113] toxicity from general anesthesia,[114], and chemotherapy,[115] which would be expected to aggravate any underlying neuropathology; moving an intrinsically sub-clinical neuronal dysfunction to an overt neurodegenerative process (figure 1c). Clearly, much more work is needed in this area, since a better understanding of these added stressors may lead to better preventive measures.

Although RNA toxicity appears to be the dominant mechanism for pathogenesis in FXTAS, several alternative models have been proposed. First, the RNA may trigger a conformational transition in one or more proteins that harbor prion-like domains, leading in turn to a propagating protein aggregate along the lines of amyloid plaque formation in Alzheimer's disease.[86, 116] In this regard, King et al.[116] noted that hnRNP A2/B1, which is found in the intranuclear inclusions of FXTAS,[18, 31, 91, 92] ranks among RNA recognition motif proteins as highly "prionogenic"; it has a high potential to form prion-like protein aggregates. By analogy with the prionogenic proteins, TBP43 and FUS, which form aggregates in the degenerating motor neurons in amyotrophic lateral sclerosis (ALS), King et al.[116] proposed that the expanded CGG repeat in the *FMR1* mRNA might trigger such an aggregation of hnRNP A2/B1. However, in contrast to the exclusively intranuclear inclusions of FXTAS, essentially all of the prionogenic proteins listed by King et al.[116] form cytoplasmic aggregates,(see also:[86]) which have never been observed in FXTAS patients. Moreover, in a *Drosophila* model of FXTAS that displays neuronal degeneration, and nuclear and cytoplasmic inclusions, overexpression of hnRNP A2/B1 actually suppresses the neurodegenerative phenotype.[92]

A second issue related to the pathogenic mechanism in FXTAS is what role, if any, is played by lowered FMRP levels in the upper premutation range. Low FMRP levels cannot be the driving factor in premutation-associated disease, since FXTAS and FXPOI are not present in the fully methylated full-mutation range, where FMRP levels are markedly reduced or absent. Moreover, Hashem et al.[117] demonstrated that CGG expression, as RNA, is necessary and sufficient to produce the characteristic intranuclear inclusions of FXTAS and the associated neuronal cell death, under conditions where FMRP levels are unchanged from normal wild-type levels. Notwithstanding the fact that FMRP levels are unlikely to lead to the neurodegenerative phenotype, lowered FMRP levels could still contribute to the severity of premutation-associated disease, particularly in those domains of clinical involvement that relate to cognitive and/or behavioral dysfunction.

Neuropathology

One of the hallmarks of FXTAS is the presence of intranuclear inclusions in neurons and astrocytes throughout the CNS of affected individuals.[18, 118] The inclusions are tau- and synuclein-negative, but do contain *FMR1* mRNA[119] and several additional proteins that, when sequestered, are expected to exacerbate cellular dysregulation and thus potentiate FXTAS.[97] Although women and men with FXTAS possess similar numbers of neuronal

inclusions, women appear to have far fewer astrocytic inclusions for reasons that are not understood at present.[85] There is a strong, positive correlation between CGG-repeat number and inclusion load,[31] and an earlier onset of the neurodegenerative disorder.[32, 120] Surprisingly, given the presumed CNS basis of the disorder, more recent studies have revealed the presence of the intranuclear inclusions throughout the PNS, including the paraspinal ganglia, pericardiac ganglia, and myenteric plexus.[121, 122] Intranuclear inclusions are also observed in the adrenal gland (cortex and medulla), pituitary, islets of Langerhans, thyroid, heart, and testicular Leydig cells.[60, 121, 122] The presence of intranuclear inclusions throughout the PNS and in multiple non-nervous tissues raises two important points regarding FXTAS pathogenesis: First, their broad distribution indicates that the mechanism(s) leading to FXTAS and, presumably, the broader range of premutation phenotypes, are operating outside of the CNS. For instance, autonomic dysfunction (e.g., orthostatic hypotension, impotence), commonly occurring before the onset of tremor or balance problems, may reflect inclusions in pericardial ganglia and testicular Leydig cells, respectively.[60] Similarly, inclusions in the cardiac tissue and pericardial ganglia likely reflect a disease process that results in cardiac arrhythmias.[122] Moreover, FXPOI could arise as a direct consequence of dysfunction in critical peripheral tissues (e.g., HPA axis).[122] Second, the presence of inclusions indicates that the core elements of cellular dysregulation are not limited to the CNS, and therefore may be studied in samples and cultured cells from those tissues (e.g., fibroblasts).[123]

Neuropathology reports for FXTAS cases have been helpful in gaining an understanding of the co-occurrence of other disorders, including Lewy body dementia, Alzheimer's disease, and multiple sclerosis.[18, 85, 111, 124, 125] In particular, the added cell stress associated with a second CNS disorder would be expected to exacerbate the cellular dysregulation that potentiates FXTAS, as through commonalities of mitochondrial dysfunction (figure 1b).[106–108] Going forward, investigation of common/overlapping neuropathological mechanisms between FXTAS and other CNS disorders will increase our shared understanding of the variability of disease expression and progression in both FXTAS and the other disorders.

Expanded Spectrum of Clinical Involvement Associated with the Premutation

Our understanding of the core and associated features of FXTAS has expanded to include a spectrum of neurological, psychological, endocrine, and immune-related characteristics that occur with increased frequency in FXTAS cases (denoted by an asterisk (*) in table 2); and other symptoms that are commonly seen in those with FXTAS have not yet been assessed in comparison to age-matched controls. Moreover, carriers without clinical evidence of FXTAS may also have some of these features, though less frequently than those with FXTAS.[49] Thus, an emerging view of FXTAS is that it is one end of the spectrum of clinical involvement that spans the entire age range. The core neurological features originally described in FXTAS occur in approximately 40% of male carriers,[126] and in approximately 16% of female carriers over the age of 50 years,[13, 49, 69] although penetrance has been less well documented in women.[72, 126–128] In both men and women, penetrance increases with age. Women with FXTAS have less white matter disease and brain atrophy on MRI, and less dementia in late-stage FXTAS than men.[19, 84] One important caveat to the above percentages is that they were generally ascertained through families with known FXS probands, which biases the allele size-distribution toward larger expansions, and hence greater propensity for clinical involvement of premutation carriers.[129]

Precisely who will develop FXTAS and/or other forms of premutation clinical involvement is likely related to background genetic predisposition and/or negative environmental factors

(figure 1c) such as environmental toxins, chemotherapy, drug and alcohol abuse, and surgery.[113, 115] Chemotherapy can exacerbate white matter disease and FXTAS symptoms,[115] and women who smoke develop FXPOI earlier than non-smokers,[7] likely due to additional oxidative stress. Based on our observations, there appears to be a more rapid deterioration of FXTAS symptoms in those addicted to alcohol or narcotics, in some instances due to addiction to opiates that were originally used to control neuropathic pain or fibromyalgia; narcotics have been shown to exacerbate white matter disease.[130–132] Even pain control with marijuana can exacerbate white matter disease.[133] The onset of FXTAS often occurs after a prolonged general surgery in aging premutation carriers in our experience. Some types of general inhalant anesthesia are intrinsically toxic to neurons,[114] and may therefore exacerbate the underlying neuronal pathology associated with FXTAS.

Immune-mediated disorders are more common in premutation carriers than controls, particularly hypothyroidism and fibromyalgia.[49, 68, 69, 71] In a study of 55 women carriers with FXTAS, 72·3% had at least one immune mediated disorder compared to 46·5% of women carriers without FXTAS and 31·5% of age-matched controls.[68] The estimated odds ratio (OR) for immune mediated disorders was 2·6 (95% CI 1·2–5·6,*P*=0·015) for women with FXTAS relative to premutation carriers without FXTAS; the OR of an immune mediated disorder in carriers with or without FXTAS was also significantly higher than for controls (OR 2·1, 95% CI 1·1–4·2,*P*=0·034; OR 5·5, 95% CI 2·4–12·5,*P*<0·001, respectively). Often features of an immune mediated disorder are present before the development of FXTAS, although it is unclear how the preceding immune mediated disorder may influence the subsequent development of FXTAS. Untreated hypothyroidism can certainly exacerbate cognitive deficits; therefore, routine screening for hypothyroidism is recommended by the primary health care provider in aging women with the premutation with or without neurological symptoms.[49] Autonomic dysfunction as an associated feature of FXTAS is now more readily interpretable as a consequence of involvement of the PNS, following the observation of inclusions within PNS neurons.[121, 122] The need for a pacemaker is not uncommon among those with FXTAS, due in part to the associated bradycardia, itself a consequence of involvement of pericardial ganglia and/or cardiac conduction system. However, pacemakers can interfere with confirmation of FXTAS, since their presence is a contraindication for MRI, which provides a major diagnostic criterion for FXTAS (table 1).[3] Moreover, gastrointestinal symptoms (e.g., constipation) may be related to peripheral disease within the myenteric plexus.

Hypertension, initially reported in 60% of women with FXTAS,[49] has subsequently been found to be significantly increased in men with FXTAS (OR=3·22; *P*=0·0003) relative to controls.[61] Although there was a trend toward hypertension in male carriers without FXTAS, the observation has not reached significance. The Framingham Heart Study recently reported associated white matter findings related to hypertension.[134] This study found that an increased systolic blood pressure was linearly associated with decreased regional fractional anisotropy and increased mean diffusivity, especially in the anterior CC, the inferior fronto-occipital fasciculi, and the fibers projecting from the thalamus to the superior frontal gyrus. Hypertension was also strongly associated with reduced gray matter volumes. Therefore, to avoid an exacerbation of white matter disease in individuals with the premutation, hypertension should be aggressively diagnosed, treated, and monitored.

FXTAS-associated psychiatric problems often begin before the emergence of tremor and ataxia in those who develop FXTAS.[10, 11, 14, 66] The increased lifetime prevalence of mood disorders (65%) and of anxiety disorders (52%) is increased in those with FXTAS.[11, 66]

Treatment of FXTAS

Currently, targeted treatments for FXTAS that can reverse the features of this disorder have not been found. However, there are several symptom-directed treatments that can be helpful for those with FXTAS[58, 72, 135] and for family members who are impacted by the level of care needed for these patients.[136] The irritability and outburst behavior that is common in FXTAS takes a significant toll on the care providers, who are usually spouses.[136] Family caregivers often become depressed and need treatment and counseling.[136, 137] Treatment of depression is of particular importance, since it is common in FXTAS and untreated depression in elderly can lead to further cognitive decline.[58, 72, 138]

There are several anecdotal reports of partial responses for the symptomatic treatment of intention tremor in FXTAS, including the use of beta-blockers, primidone, or topiramate.[2, 58, 72] Deep brain stimulation also was reported to be beneficial for severe intention tremor of FXTAS in two reports,[139, 140] but this method also exacerbated ataxia in two of three cases in the latter report. Botulinum toxin A injections have also been reported to be helpful for the treatment of tremor in FXTAS.[72] Current treatments for ataxia are not generally effective, though case reports have reported some benefit from riluzole, varenicline, amantadine, or buspirone.[72, 141] Physical therapy may also be indicated to improve strength and stability in those with ataxia, although a controlled study has not been performed.[58] Interventions for cognitive decline in FXTAS have included memantine[142] and anticholinesterase inhibitors,[72] with case reports describing positive results. Venlafaxine has been reported to help with attention and executive function deficits, and improved depression in a several cases, but a controlled trial has not been carried out.[72, 142]

Treatment for the underlying RNA toxicity of FXTAS will require further study. The observation that allopregnanolone normalizes network activity in neurons cultured from premutation mice,[35] coupled with the observed $GABA_A$ deficit in FXTAS patients,[36] suggests that a $GABA_A$ agonist will be beneficial to those with FXTAS. Moreover, at the cellular level, an mGluR5 antagonist similarly improved neuronal network activity,[35] pointing to an excitatory/inhibitory imbalance that is reminiscent of FXS. Currently, both GABA agonists and mGluR antagonists are being studied in individuals with FXS.[143, 144] Given the significant oxidative stress in cells harboring premutation alleles,[35, 106, 107, 112] it follows that antioxidants should be of some benefit as an adjunct to the treatment of FXTAS; however, no controlled trials have been completed for antioxidants or other specific treatment/intervention for FXTAS or, more broadly, for any form of premutation involvement. Clearly, there is an acute need for additional treatment studies. One potential approach for direct targeting of the underlying pathogenic mechanisms giving rise to FXTAS would be to use synthetic oligonucleotides to degrade the expanded CGG-repeat mRNA, thus eliminating the RNA “trigger”. Since the site of action of the CGG-repeat–containing mRNA is likely to be intranuclear, oligonucleotides that operate within the nucleus would be required. One encouraging example of this approach has involved the use of a synthetic “gapmer” oligonucleotide for targeted knockdown, both in culture and in an *in vivo* mouse model involving direct intramuscular delivery, of the CAG-repeat *DMPK* RNA associated with DM1.[145] Although this type of approach holds great potential for the treatment of FXTAS, substantial barriers remain. In particular, since FXTAS is principally a CNS disorder, delivery of the oligonucleotides across the blood-brain-barrier would be required.

Reconceptualizing and Redefining FXTAS

Since the initial definition of FXTAS focused on the core features of progressive tremor and ataxia, and associated cognitive decline, premutation-associated features that are not

manifestly progressive (e.g., emotional, behavioral disturbances) should not be considered part of the FXTAS diagnosis, even though they may involve the same underlying pathogenic mechanism(s) as the neurodegenerative phenotype. Furthermore, symptoms of intermittent numbness and tingling, immune mediated disorders, or FXPOI, which are very common in carriers with and without FXTAS,[49] cannot currently be said to presage FXTAS.

Given the nearly universal presence of neuronal dysfunction in the neonatal premutation mice, we propose that there is a state of cellular dysfunction (e.g., altered mitochondrial function; disturbed calcium regulation) among carriers that potentiates the adult-onset clinical involvement and eventual neurodegeneration, but which may not be sufficient for an overt phenotype in the absence of one or more additional genetic and/or environmental factors (figure 1c). Thus, symptoms of FXTAS may be precipitated by trauma, severe illness (cancer and chemotherapy;[115]), prolonged surgery with general anesthesia or exposure to toxins,[113] genetic predisposition for mitochondrial dysfunction,[106, 107] or the coexistence of another disease such as multiple sclerosis[49, 110, 111] or Alzheimer's disease.[85] In this regard, Cornish et al.[146] have identified a set of neuropsychological deficits, including executive function deficits and increasing impulsivity, in older carriers that may portend FXTAS. Moreover, Yang et al.[77] have reported a subgroup of individuals with the premutation with increasing dysfunction in DTI studies involving fronto-cerebellar circuits, which may also point to those with the highest risk for FXTAS. However, for both of these investigations, longitudinal studies are still needed to assess these approaches for their ability to predict who will develop FXTAS – a critical undertaking for enabling early treatment for those who will eventually develop the neurodegenerative disorder.

Finally, although FXTAS has been framed exclusively as a premutation-associated disorder, the neurodegenerative features of FXTAS have been observed in a small number of individuals with an unmethylated full-mutation allele,[147] presumably a consequence of continued production of elevated levels of *FMR1* mRNA,[148] and in some with a gray zone allele (45 to 54 CGG repeats).[73, 149] In addition, small intranuclear inclusions associated with FXTAS have been reported in three males with full mutation alleles.[59] Loesch et al.[147] describe a 65-year-old man with a full mutation (247–480 repeats) that was completely unmethylated, with a 3·5-fold elevation of *FMR1* mRNA. This individual was a truck driver with a borderline IQ who developed alcoholism at age 54, followed by memory deficits, type II diabetes, stomach cancer surgery, worsening of tremor and ataxia, and eventually dementia. His MRI was classic for FXTAS, including the MCP sign, and white matter involvement of the pons, CC, and periventricular area.

Clearly, the current diagnostic criteria for FXTAS need to be updated, and should include the possibility that individuals with CGG-repeat expansions that lie outside of the premutation range can develop this disorder. The broader issue of how to define included/excluded clinical features will also need to be revisited. In 2013, revisions to the diagnostic criteria (table 3) will be reviewed by an international group of experts to develop a global consensus for the revised criteria.

Conclusions and Future Research Directions

FXTAS represents the severe end of premutation-associated disorders, which include FXPOI, developmental delays, autism spectrum disorder, ADHD, anxiety, depression, and a variety of medical/neurological problems, such as migraines, fibromyalgia, neuropathy, sleep apnea, hypertension, and hypothyroidism. These problems are related to RNA toxicity caused by elevated levels of *FMR1*-RNA in premutation carriers. Future research directions should focus on variable penetrance of clinical involvement, and the environmental and genetic factors that can precipitate or prevent clinical involvement. Except for a current

controlled trial of memantine, FXTAS-directed treatment approaches have not been investigated. Future directions of treatment should be driven by molecular advances regarding the triggering mechanism of FXTAS, and by targeted treatments that have the potential to halt or reverse the neurodegeneration of FXTAS. Prophylactic interventions that protect the brain from white matter changes and from oxidative stress should be studied. Because the premutation is common in the general population and most carriers will be affected with at least one premutation-associated disorder, we recommend that clinicians test for the premutation—by ordering fragile X DNA testing—when one of the premutation disorders is present, particularly when symptoms of tremor, ataxia, or primary ovarian insufficiency are observed. Genetic counseling for the family is recommended if a carrier is identified.[136]

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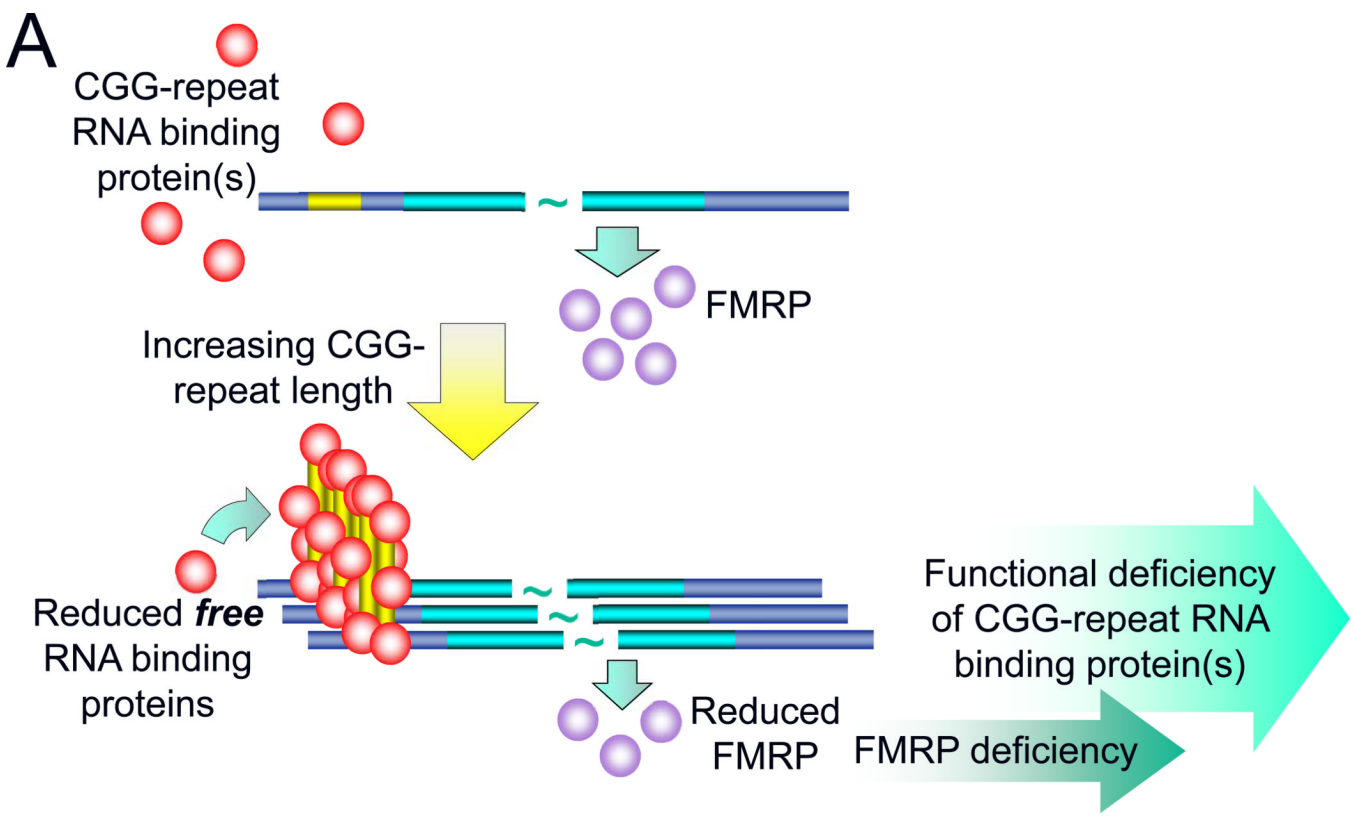

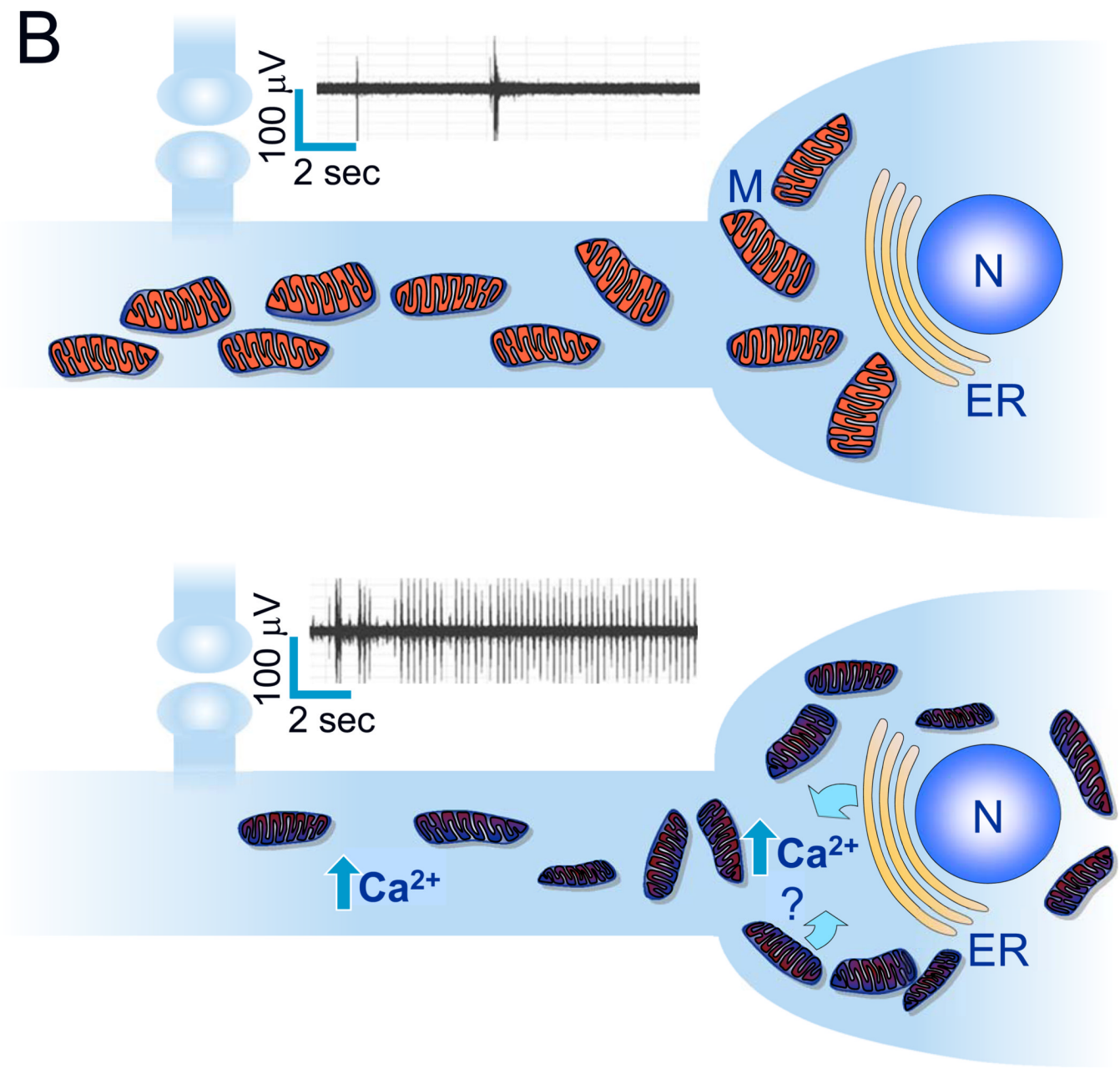

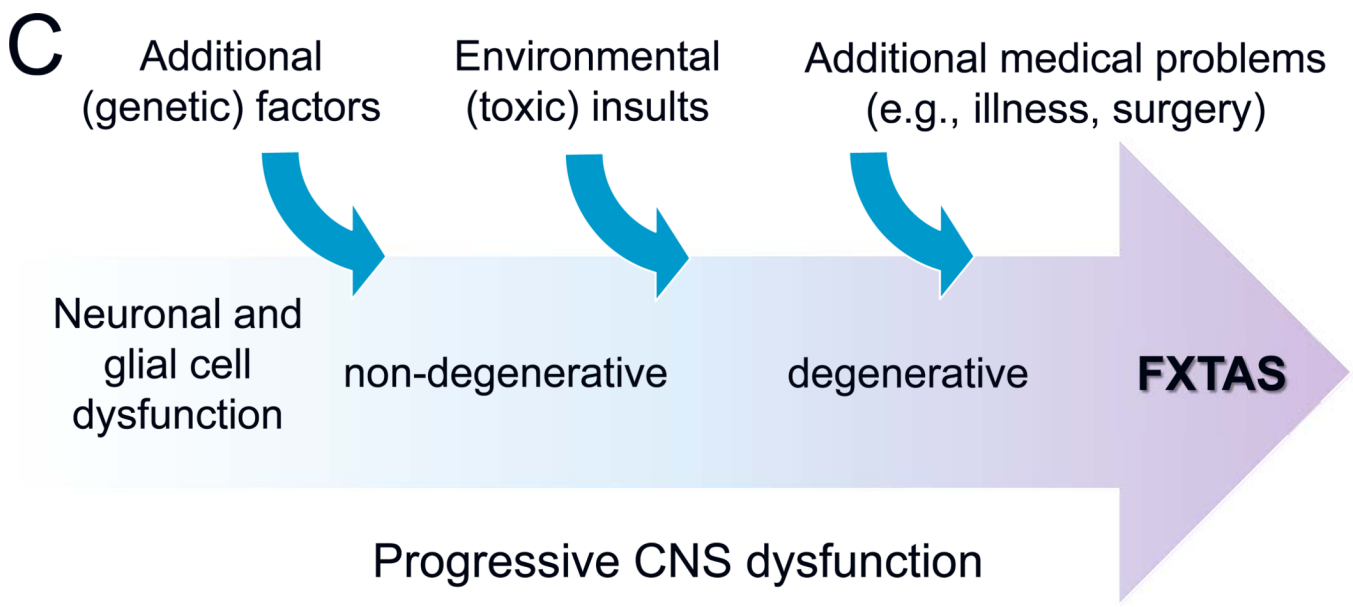

Figure 1.

(**A**) Schematic of the sequestration model for RNA toxicity in the fragile X premutation disorders, including FXTAS. One or more RNA-binding proteins bind to the CGG-repeat RNA in a length-dependent fashion, such that little binding occurs in the normal CGG-repeat range. Excess binding/sequestration of those proteins leads to a functional insufficiency for their normal function(s). Reduced *FMR1* levels may contribute to the premutation phenotypes for the larger premutation alleles. (**B**) Stylized neuron with single synapse represented. CGG-repeat expansion leads to downstream effects that include reduced mitochondrial function, altered calcium regulation, and increased, synchronous firing of neurons in mouse hippocampal neuronal networks. (**C**) Schematic representation of the progression of CNS dysfunction, primarily driven by the premutation CGG-repeat expansion, but also modulated by second-gene effects, and various environmental exposures (e.g., untreated hypertension or hypothyroidism, smoking and/or use of other agents that promote oxidative damage, major illness or – anecdotally – surgery requiring general anesthesia).

Table 1

Current diagnostic criteria for FXTAS[4,41]

Examination and Degree	Observation
Molecular	
Major	55–200 CGG repeats (premutation)
Radiological	
Major	MRI white matter lesions in MCPs
Minor	MRI white matter lesions in cerebral white matter
Minor	Moderate-to-severe generalized atrophy
Clinical	
Major	Intention tremor
Major	Gait ataxia
Minor	Parkinsonism
Minor	Moderate-to-severe short-term memory deficiency
Minor	Executive function deficit
Neuropathological	
Major	FXTAS inclusions

FXTAS Diagnostic Categories

Category	Description
Presence of expanded CGG repeat (molecular), ***and***	
Definite	Presence of one major radiological sign *plus* (i) one major clinical symptom *or* (ii) the presence of FXTAS inclusions.
Probable	Presence of one major radiology sign ***and*** one minor clinical symptom, ***or*** two major clinical symptoms.
Possible	Presence of one minor radiology sign ***and*** one major clinical symptom.

Table 2

Established and expanded features of FXTAS

Autonomic problems	
constipation/irritable bowel syndrome	Hagerman et al. (2008);[58] Hunsaker et al. (2011)[59]
erectile dysfunction	Greco et al. (2007)[60]
problems swallowing	Leehey et al. (2007)[40]
gastrointestinal reflux	Leehey et al. (2007)[40]
orthostatic hypotension	Leehey et al. (2007)[40]
hypertension*	Coffey et al. (2008);[49] Hamlin et al. (2012)[61]
urinary urgency & incontinence	Leehey et al. (2007);[40] Leehey et al. (2011)[62]
cardiac arrhythmia	Leehey et al. (2011)[62]
dizzy spells or vertigo	Leehey et al. (2011)[62]
Sensory	
olfactory dysfunction*	Juncos et al. (2012)[57]
hearing loss*	Juncos et al. (2011)[5]
neuropathy*	Berry-Kravis et al. (2007);[50] Jacquemont et al. (2003);[4] Hagerman et al. (2007);[48] Soontarapornchai et al. (2008);[63] Juncos et al. (2011)[5]
Sleep problems	
insomnia*	Chonchaiya et al. (2010)[64]
sleep apnea*	Hamlin et al. (2011)[65]
daytime sleeping	Hamlin et al. (2011)[65]
Motor symptoms	
tremor+,*; ataxia+,*	Hagerman et al. (2001);[3] Jacquemont et al. (2003);[4] Juncos et al. (2011);[5] Apartis et el. (2012)[2]
muscle weakness	Leehey et al. (2007)[40]
parkinsonism+,*	Jacquemont et al. (2003);[4] Healey et al. (2009);[52] Hall et al. (2010);[55] Madeo et al. (2012)[54]
Psychiatric	
depression*; anxiety*	Bourgeois et al. (2011);[11] Seritan et al. (2013)[66]
irritability	Bacalman et al. (2006)[67]
Chronic pain	
fibromyalgia*	Winarni et al. (2012);[68] Rodriguez-Revenga et al. (2009);[69] Coffey et al. (2008);[49] Leehey et al. (2011);[70] Apartis et al. (2012)[2]
neuropathic pain*	
Immune-mediated disorder	
hypothyroidism*	Winarni et al. (2012);[68] Rodriguez-Revenga et al. (2009);[69] Coffey et al. (2008);[49] Leehey et al. (2011);[70] Behm et al.[71]

	fibromyalgia*

*Features that occur in a greater number of those with FXTAS compared to age-matched controls.

+Established features.

Table 3

Suggested modifications to FXTAS diagnostic criteria presented in table 1

Examination and Degree		Observation
Molecular		
	Major	Expand from premutation range to include - 45–54 CGG repeats (gray zone) - full mutation with continued production of *FMR1* mRNA
Radiological		
	Major	Expand to include MRI white matter lesions in pons, corpus callosum, and/or insula
Clinical		
	Minor	Expand to include peripheral neuropathy
Neuropathological		
	Major	FXTAS inclusions