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Fragile X-associated tremor/ataxia syndrome

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Abstract

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that affects some but not all carriers of small, non-coding CGG-repeat expansions (55–200 repeats; premutation) within the fragile X gene (*FMR1*). Principal features of FXTAS include intention tremor, cerebellar ataxia, Parkinsonism, memory and executive function deficits, autonomic dysfunction, brain atrophy with white matter disease, and cognitive decline. Although FXTAS was originally considered to be confined to the premutation range, rare individuals with a gray zone (45 to 54 repeats) or an unmethylated full mutation (>200 repeats) allele have now been described; the constant feature of the disorder remaining the requirement for *FMR1* expression, in contradistinction to the gene silencing mechanism of fragile X syndrome. Although transcriptional activity is required for FXTAS pathogenesis, the specific trigger(s) for FXTAS pathogenesis remains elusive, highlighting the need for more research in this area. This need is underscored by recent neuroimaging findings of changes in the central nervous system that consistently appear well before the onset of clinical symptoms, thus creating an opportunity to delay or prevent the appearance of FXTAS.

Keywords

neurodegeneration; dementia; premutation; RNA toxicity; CGG repeat; FXTAS

Background

Over the last decade, our understanding of fragile X-associated disorders (FXD), which arise from full mutation (>200 CGG repeats in the gene *FMR1*; fragile X syndrome) or premutation (55 to 200 CGG repeats) alleles, has dramatically evolved. Although

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Conflicts of Interest

R. Hagerman has received funding from Novartis, Roche, Seaside Therapeutics, Alcobra, and Neuren to carry out treatment studies in fragile X syndrome, autism, or Down syndrome; he has also consulted with Novartis and Roche/Genentech regarding treatment studies in fragile X syndrome. P. Hagerman is an uncompensated collaborator with Pacific Biosciences regarding new *FMR1* sequencing strategies; he holds patents for *FMR1* genotyping and protein tests.

premutation disorders were once thought to include only fragile X-associated primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS), we now know that carriers of a premutation allele have a variety of medical problems that include psychiatric disorders of anxiety and depression;[1–5] chronic pain syndromes, such as fibromyalgia[6–8] and chronic migraine;[9] hypothyroidism;[4,7] hypertension;[10] sleep apnea;[11] vertigo, olfactory dysfunction, and hearing loss;[4,12,13] and enhanced stress.[14] In addition, some premutation carriers have neurodevelopmental disorders, such as intellectual disability[15] and/or autism spectrum disorder (ASD).[16,17] Although most of the premutation problems are thought to relate to elevated *FMR1* mRNA,[18,19] there is also evidence of FMRP deficits adding to the phenotype, especially when significant cognitive deficits are present; FMRP mRNA levels are lowest in the upper premutation range.[20–22] ASD in premutation carriers is also related to the presence of seizures.[17] Early life seizures cause FMRP to redistribute from the dendrites to the cell body, rendering FMRP incapable of properly regulating translation at the synapse.[23] Therefore, early life seizures can impede development as a consequence of a functional insufficiency of FMRP at the synapse.

Additional factors can influence the phenotype of premutation carriers. In approximately 20% of premutation cases with ASD or neurological problems, a second genetic hit has been identified through either microarray testing or whole exome sequencing.[24] Such second hits are thought to contribute to the penetrance and/or severity of the phenotype, thus compounding intellectual disability, ASD, or neurological problems. Environmental toxicity can also cause additive effects to the premutation phenotype because premutation neurons are more vulnerable to toxic insults than are control neurons.[25] Specifically, exposure to environmental toxins can lead to a more severe phenotype or earlier onset of FXTAS.[26] In this regard, chemotherapy for cancer has been observed to precipitate FXTAS.[27] In addition, some patients have reported that surgery involving general anesthesia leads to onset of tremor or ataxia within weeks in those carriers over 60 years of age, suggesting that one or more of the agents used during general anesthesia, or perhaps the surgical procedures themselves (e.g., hypoxia, tissue damage), may exacerbate the premutation-associated disorder. Unfortunately, essentially all of our awareness of a possible association between general surgery and FXTAS is based at present on anecdotal information, underscoring the need for systematic studies in this area.[28]

Expanding the diagnostic criteria for FXTAS

The standard diagnostic features of FXTAS require a premutation *FMR1* allele plus one or more of the following core diagnostic features: intention tremor, cerebellar ataxia (core neurological features), and white matter disease in the middle cerebellar peduncles (MCP sign).[29] Additional features contributing to the diagnosis include executive function and memory deficits, Parkinsonism, and additional MRI findings of global brain atrophy and white matter disease.[4,12, 22, 30–33]

However, recent cases of FXTAS, identified through core diagnostic features, have been found among carriers of gray-zone alleles (45–54 CGG repeats),[34,35] and, in rare cases, among those with unmethylated, full mutation or mosaic alleles.[34,36–38] These observations underscore the need to develop a broader definition of the disorder, since elevated mRNA

and RNA toxicity are expected even outside of the premutation range when mRNA levels are elevated.[19]

The diagnostic criteria for FXTAS developed in 2003 (Ref. 30) were reviewed by an international research and clinical consortium in 2013, which gave specific recommendations regarding expanding the diagnostic criteria for FXTAS. These recommendations are summarized in Hall *et al*.[33] and include broadening the range of the CGG repeat to incorporate gray-zone alleles on the low end and unmethylated/mosaic full-mutation alleles with elevated mRNA on the high end. In addition to the middle cerebellar peduncle (MCP) sign, several researchers have seen corpus callosum changes, including thinning and white matter disease in the splenium, in approximately 60% of patients, similar to the prevalence of the MCP sign in males with FXTAS.[22, 32] Lastly, neuropathy, which is more common in premutation carriers with FXTAS compared to age-matched controls,[32, 39] has been added to the diagnostic criteria as a minor sign, rather than a major criterion, since it is common in the aging population.

Several additional medical problems that are more common in carriers than controls include immune-mediated disorders, hypertension, autonomic dysfunction, sleep apnea, hearing loss, and migraines, may also occur in those with FXTAS, but typically start before its onset.[22]. Wheeler *et al*.[4] carried out a detailed review of all premutation studies to assess the strength of symptom association with the premutation in women. Their review, in addition to the report by Hall *et al*.,[33] represents consensus statements from the 1st International Conference on *FMR1* Premutation: Basic Mechanisms and Clinical Involvement, held in Perugia, Italy, in June 2013.

A clear message from premutation research is that forms of clinical involvement occur throughout the life of the carrier—with deficits in visual perceptual abilities in infancy;[40] common problems of attention, anxiety, and social interactions in childhood;[16,17] psychiatric problems, migraines, hypothyroidism, hypertension and immune-mediated problems in adulthood;[4,6, 22, 41,42] and onset of additional medical problems in a significant percentage in aging carriers of the premutation, including neuropathy, pain symptoms, and FXTAS.[32,33] Most individuals with the premutation have normal intellectual abilities and often have productive and successful lives until their 60s, when subsequently approximately 40% of males and 16% of females develop FXTAS.[7, 43]

Why some individuals develop FXTAS and other do not may have to do with additional genetic hits (e.g., the ApoE4 allele[44]) that are associated with FXTAS, which may include Alzheimer disease.[45] Forms of environmental toxicity can also add to the earlier onset or severity of FXTAS, and include smoking, alcoholism, and chemotherapy; or untreated medical problems, such as hypertension, depression, stress, hypothyroidism, cardiac arrhythmia, metabolic syndrome, or sleep apnea with hypoxia.[28] However, the problem remains that we do not know who will eventually develop the neurodegenerative disorder. Regarding markers of early processes that will eventually lead to FXTAS—or may be indicators predictive of its eventual onset—neuroimaging studies have demonstrated CNS changes well before the onset of clinically diagnosed FXTAS. These changes are visible as

functional MRI (fMRI), diffusion tensor imaging (DTI), and/or fiber track changes[46–50] and brain atrophy, particularly in the cerebellum.[51,52]

Emerging concepts about the molecular mechanism(s) leading to FXTAS

Protein sequestration by the FMR1 CGG-repeat mRNA

Perhaps the singular feature of FXTAS relating to its underlying mechanism is that its phenotypic expression is largely limited to carriers of premutation alleles—and, rarely, to individuals with size- or methylation-mosaicism[36–38,53] (however, see Ref. 54) where *FMR1* is transcriptionally active. The requirement for mRNA expression has led to a hypothesized mechanism of pathogenesis that involves a toxic gain-of-function of the expanded CGG-repeat mRNA.[55–57] As currently envisioned, the gain-of-function arises through the adventitious binding/sequestration by the CGG repeat of one or more proteins, thus at least partially impairing their normal function in the cell. This proposed mechanism is based on an analogous process operating in myotonic dystrophy, where a CUG-repeat expansion in the 3′ UTR of the myotonic dystrophy (DM1) protein kinase gene (*DMPK*) (or a CCUG repeat in the case of DM2;[58, 59]) sequesters the splice modulator, muscleblind-like 1 (*MBNL1*), resulting in altered splicing of proteins that underlie the major phenotypic domains of DM.[60–62] For the sequestration model of FXTAS pathogenesis, a number of candidate proteins exist based on evidence for CGG-repeat RNA binding and studies *in vitro* and *in vivo* in various animal models (Table 1).

All of the sequestration models share a common theme; namely, that the normal function(s) of the candidate protein is diminished through the process of sequestration, with the nature and severity of phenotypic involvement in FXTAS expected to arise through simple insufficiency of the sequestered proteins. For the proteins listed in Table 1, there is evidence of the necessary protein–CGG-repeat RNA interactions (at least *in vitro*) and the expected functional insufficiency; for example, altered splicing due to lowered Sam68;[63,64] and reduced levels of mature miRNAs due to loss of DGCR8/Drosha activity.[65]

Further, for the specific sequestration mechanisms listed in Table 1, and others not yet identified, two issues should be borne in mind. First, although each of the listed proteins has support from *in vitro*, *in cellulo*, and, in some instances, *in vivo* (*Drosophila*) data (e.g., Purα;[66] hnRNPA2[67]), there are virtually no studies of the consequences to partial depletion of functional protein and, therefore, of the specific role of the protein(s) to the pathogenesis of FXTAS. In this regard, reversal of the model phenotype by the expression of a superabundance of protein (to replace the sequestered species) may reflect mechanisms that are unrelated to the original insufficiency, as might occur through a chaperone function of the added protein. Second, although individual studies have generally focused on individual proteins (e.g., Purα, Sam68, etc.), more than one protein may likely be contributing to the overall disease phenotype. Thus, no single protein candidate for sequestration needs to be responsible for all of the phenotypic domains of FXTAS (e.g., movement disorder, cognitive impairment). In many cases other diseases have also occurred with FXTAS, including multiple sclerosis,[68] Lewy body dementia,[69] Parkinson disease,[70] and Alzheimer disease.[45]

Reduced FMRP plays only a secondary, non-causative role in the FXTAS phenotype

We and others have observed that FMRP levels trend downward with increasing CGG repeats in the premutation range,[21, 71] suggesting that mild-to-moderate FMRP insufficiency is contributing to the reduced cognitive strength observed in a portion of premutation carriers well before the typical age of onset of FXTAS.[72–74] Those who have neurodevelopmental problems prior to the onset of FXTAS may be predisposed to an earlier onset of FXTAS[70] (and Basuta *et al*., unpublished results); however, there is no evidence to date suggesting that lowered FMRP levels are causative of FXTAS, and nearly all individuals with fragile X syndrome (with little or no FMRP) do not develop FXTAS.

Antisense FMR1 RNAs

Mounting evidence shows that multiple antisense transcripts are produced from *FMR1* in a manner that tracks with the size of the CGG-repeat and, consequently, with the level of expression of the primary sense transcript, which is elevated in the premutation range and generally absent in the full-mutation range,[75–77] suggesting that the CGG repeat is capable of regulating bidirectional transcription at the locus. Interestingly, there is a specific antisense splice isoform that is only found with transcripts from premutation alleles,[75] raising the possibility that Sam68 and/or MBNL1, both of which are associated with the CGG repeat, could be functionally dysregulated by the expanded CGG repeat.

Repeat-associated non-AUG (RAN) translation

A model for FXTAS pathogenesis has been proposed in which “toxic” peptides are generated by initiating at non-AUG codons located upstream of the CGG-repeat element (Fig. 1);[78] RAN translation has been well described in other trinucleotide-repeat disorders.[79] In the current instance, Todd *et al*.[78] have provided ample evidence that RAN translation of the *FMR1* mRNA generates a poly-glycine peptide that is toxic to cells and is detectable in both the intranuclear inclusions of FXTAS and in the inclusions of the Dutch premutation CGG-repeat mouse model (see Refs [19] and [78]). However, the role of the poly-glycine peptides in FXTAS remains an open question, given that a highly related mouse model, termed the “NIH” model,[78, 80] displays significant neurodegeneration but apparently does not produce the poly-glycine peptide because of a stop codon just downstream of the initiating codon for the that peptide.[78] These seemingly paradoxical observations may indicate that the nuclear inclusions are themselves a consequence of RAN translation, but that the FXTAS-associated neurodegeneration is due to other mechanisms—this is an important area for further study.

R-loop formation/DNA damage response

All of the foregoing models for FXTAS pathogenesis—aberrant (RAN) translation, generation of premutation-specific antisense RNAs, and sequestration of one or more RNA binding proteins by the CGG-repeat element in the mRNA—are based on the involvement of the *FMR1* mRNA at the post-transcriptional level. However, the requirement for *FMR1* mRNA production does not preclude the involvement of co-transcriptional processes (Fig. 1). In this regard, transcription through the highly GC-rich *FMR1* 5′ UTR region promotes (co-transcriptional) R-loop formation,[76] whereby the G-rich RNA transcript reinvades the

DNA duplex and forms a stable RNA:DNA hybrid with the C-rich template strand, thereby displacing the non-template DNA strand. Although R-loop formation normally occurs at multiple loci throughout the genome,[81] defects in mRNA processing can result in an R-loop–dependent activation of the DNA damage response and lead to the accumulation of the phosphorylated H2A variant, γH2AX, which is associated with the DNA damage repair process.[82, 83]

We have recently reported R-loop formation both at the endogenous *FMR1* locus and in an inducible episomal system in which the expanded CGG-repeat (~95 CGG) is located upstream of a GFP reporter.[83] For the episomal system, we had previously reported that high levels of reporter mRNA with the expanded CGG repeat results in the generation of γH2AX,[84] which is also found within the intranuclear inclusions of FXTAS.[85] Thus, although indirect, current evidence suggests that the increased transcriptional activity associated with premutation alleles may lead to damage at the *FMR1* locus and a consequent DNA damage response (DDR; Fig. 1), a possibility that warrants additional study.

For all of the aforementioned mechanisms there remains the task of assessing to what extent they play a role(s) in the development of FXTAS in humans, and whether such mechanisms, singly or in combination, are either sufficient for pathogenesis or secondary (e.g., FMRP deficiency). Moreover, although mitochondrial dysfunction is clearly a component of FXTAS pathogenesis[86–89] (reviewed elsewhere, e.g. Ref. 19), neither its linkage to *FMR1* mRNA expression nor its specific role in pathogenesis is clear at present.

A nematode model for CGG-repeat–mediated alterations in adaptive behavior

An important feature of the clinical presentation of fragile X syndrome is hyperarousal and an enhanced reaction to various forms of sensory stimuli.[90–92] Enhanced response to auditory stimuli was quantified by measuring the electrodermal response (EDR) exhibited by a subject with fragile X syndrome to a defined stimulus[91] (see also Ref. 93). The EDRs were significantly enhanced in the subjects relative to controls, with increased magnitude of the response and reduced habituation (*i.e.*, a failure to adapt or attenuate the EDR with continued sensory stimulation). More recently, Schneider *et al.*[92] demonstrated a related feature in carriers of premutation alleles, including FXTAS patients. They applied a low intensity acoustic “prepulse” at varying intervals prior to a main pulse to determine to what extent the prepulse would attenuate (inhibit) the subjects’ reaction to the main pulse (prepulse inhibition, PPI). They observed an impaired PPI that was most pronounced in male FXTAS patients, with a significant correlation between CGG-repeat size and the magnitude of the PPI deficit. Thus, for both premutation and full mutation carriers, there is a failure to habituate to a sensory stimulus.

Remarkably, Juang *et al.*[94] observed an analogous loss of habituation—olfactory habituation—in a nematode (*Caenorhabditis elegans*) model in which an expanded CGG-repeat expression vector (99 CGG repeats) is transcriptionally active solely in a single pair of olfactory neurons. A noteworthy aspect of the *C. elegans* model is that it does not involve

neuronal death, unlike the neuronal death observed in the *Drosophila* CGG-repeat–coupled neurodegenerative phenotypes.[56, 66, 78, 95, 96]

Odor-seeking behavior of C. elegans involves just two pairs of ciliated olfactory neurons, AWA and AWC, to sense many chemoattractants.[97] The primary odor-sensory AWC neurons allow worms to chemotax toward attractive volatile chemicals and govern both the primary olfactory response and a secondary adaptive response, which requires neuronal plasticity.[97, 98] A single AWC neuron senses the chemoattractant butanone and directs nematodes to move toward this odor source;[99] prolonged odor exposure in the absence of food reduces the animal's attraction to butanone[100,101] because of processes that occur within the AWC neuron.[94,98,102–104] Remarkably, expression of the expanded CGG-repeat element (but not the normal CGG repeat) in only a single pair of AWC neurons interfered with nematode's ability to attenuate the prolonged exposure to the odorant, while preserving normal odor detection.[94] The authors found that the abnormal response to the expanded CGG repeat is mediated by the miRNA-specific Argonaute (ALG-2), suggesting that this pathway may play a role in the pathogenesis of neuronal function in FXTAS. However, of more immediate interest is the potential of this system for studying the detailed biochemical mechanism for altered neuronal function.

Approaches to treatment of FXTAS

One of the first steps in treating FXTAS is identifying those with neurological problems who also carry premutation *FMR1* alleles. For individuals within families with known children or adults with fragile X syndrome (FXS), identification and treatment are generally straightforward; with new options available for targeted treatments to reverse the neurobiological abnormalities that occur with the absence of FMRP.[105–108] Those with the premutation and a variety of medical problems have several options for symptomatic treatment,[28,109] including the use of selective serotonin reuptake inhibitors (SSRIs) for depression and/or anxiety.[3,41]

For those with FXTAS, a controlled trial was carried out to assess the benefit of memantine over a 1-year treatment period.[110] Although memantine was well tolerated, it did not demonstrate benefit compared to placebo in alleviating the tremor, balance problems, or executive function deficits in those with FXTAS.[110] However, a subgroup of patients who participated in the memantine study exhibited significant improvement in a secondary measure, an event-related potential (ERP) paradigm to assess verbal fluency and memory for non-congruent words with memantine.[111] Since a subgroup of patients with FXTAS treated with memantine show some improvement,[112] further studies are needed to identify this subgroup. ERPs are likely a more sensitive outcome measure for validating improvements in brain processing with targeted treatments, as has been found in treatment of FXS.[113]

Premutation neuronal cultures have demonstrated that elements of RNA toxicity, such as up-regulation of heat shock proteins and enhanced spike discharges from the neuron, are improved with the addition of allopregnanolone or even an mGluR5 antagonist, MPEP.[114] MPEP is neurotoxic in humans, but allopregnanolone is a natural neurosteroid that has neuroprotective features and stimulates neurogenesis; allopregnanolone is now being

utilized in trials of traumatic brain injury and Alzheimer disease.[115,116] Treatment trials of allopregnanolone will likely begin in the near future for those with FXTAS.

Those with premutation alleles are generally identified as obligate carriers based on the pattern of cases of fragile X syndrome or are identified through cascade testing within the family.[17,117,118] However, for isolated individuals with neurological involvement, proper identification of FXTAS cases necessitates the recognition of the phenotypic features of the disorder, which often overlap those of many other disorders with ataxia, tremor, and progressive cognitive impairment. Because of the phenotypic overlap with spinocerebellar ataxias, Parkinson disease, and dementias, screening studies have been performed to assess the extent to which such disorders are actually manifestations of FXTAS.[119–128] Results of these studies—and others—have been variable, with the most consistent yield of FXTAS cases (with premutation alleles) found among the ataxias, typically in the range of ~1–2%, with more negative results found in cases with essential tremor, Parkinson disease, Alzheimer disease,[128,129] and related disorders. Those studies with negative findings likely reflect the much higher prevalence of these latter disorders compared to FXTAS, necessitating much larger screened populations to determine whether they have any association with the premutation allele.

In a recent screening study in India of a cohort with progressive, late-onset tremor/ataxia (109 patients and 173 control subjects),[130] three premutation alleles were detected among the patient group; two of these individuals (96 and 102 CGG repeats) were being evaluated for SCA-12 and another (78 CGG repeats) for progressive gait ataxia. The frequency of FXTAS was 3.3% overall and 9% (2/23 cases) for SCA-12-like presentation, underscoring the need to screen cases of apparent (test-negative) SCA.

Differential diagnosis and testing

Features of FXTAS overlap many of the core features of other neurodegenerative disorders, including Parkinson and Alzheimer diseases and frontotemporal dementia (e.g., progressive cognitive impairment, altered mood and behavior). Therefore, it is important to consider FXTAS in the differential diagnosis of a wide range of neurological or neurodegenerative disorders (*e.g.*, Table 23.2 of Ref. 131). Such overlap also includes disorders with ataxia and/or intention tremor, such as the SCAs, multiple system atrophy (MSA), Parkinson disease (PD), essential tremor, progressive supranuclear palsy (PSP),[132] and essential tremor.

However, for most of the above-mentioned disorders, testing for a premutation allele of the *FMR1* gene would not generally be productive unless there are additional indicators pointing to that gene. In particular, testing for an expanded allele of the *FMR1* gene should be considered in patients who present with cerebellar ataxia and/or action tremor, particularly for those older than 50 years, when the core motor involvement is accompanied by one or more of the features listed in Table 2. Some of these associated conditions—family history of cognitive impairment and/or autism/ASD, primary ovarian insufficiency, family history of fragile X—are indications for fragile X testing that are independent of the presence of

motor involvement, but are clear indications for testing with an initial presentation of tremor/ataxia.

Conclusions

New treatment studies are needed for this relatively common disorder of neurodegeneration —FXTAS. The premutation occurs in 1 in 130–250 females and about half that number in men in the general population (reviewed in Ref. 133). Changes in the CNS, revealed by recent neuroimaging studies, are consistently seen in adulthood well before the onset of the clinical symptoms of FXTAS; this general observation provides the opportunity to treat the medical problems well before the age of typical onset of FXTAS, thus creating a window of therapeutic opportunity to delay or even prevent the neurodegenerative disorder.[28]

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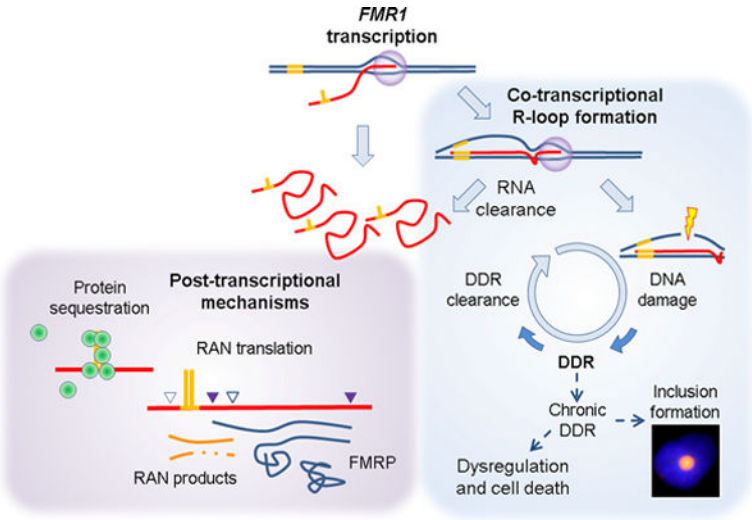

Figure 1.

Models for pathogenesis of FXTAS; all are based the requirement for expression of the expanded (premutation) *FMR1* mRNA. Post-transcriptional models are based on the properties of the expanded CGG-repeat region within the free mRNA. The CGG-repeat element, likely through its ability to form higher-order structure (e.g., hairpin; depicted in yellow), has been demonstrated to sequester one or more proteins (green spheres), rendering the cell functionally deficient in those proteins; the exemplar for this process is the muscleblind-like 1 protein of myotonic dystrophy. An alternative mechanism has been proposed in which the CGG element leads to aberrant initiation of translation, out of phase with the FMRP coding sequence, and at non-AUG codons. In this model, the short peptides are proposed to be toxic. Despite the focus on post-transcriptional mechanisms, co-transcriptional events (e.g., R-loop formation) can also lead to cellular toxicity, in this instance through failure to clear DNA damage and its repair at/near the R-loop. Although R-loop formation and DNA damage repair (DDR) are occurring normally throughout the genome, occasional failure to clear the DDR response can lead to continued signaling and recruitment of repair proteins. In this regard, we have observed the presence of the DDR signaling protein, gH2AX, in the inclusions, as well as its induction in model systems of CGG-repeat overexpression. None of these models is mutually exclusive.

Table 1

Summary of the models that have been invoked as mechanisms for FXTAS pathogenesis

Mechanism class	Initiating species	Mechanism	Role in human disease	Citations
Protein sequestration by the CGG-repeat RNA				131,134,135
	hnRNP A2/B1	Both hnRNP A2/B1 and Purα appear to bind preferentially, at least *in vitro*, to shorter (~20 CGG) repeat RNAs, which renders them less likely candidates for FXTAS pathogenesis	Have mediating effects on *Drosophila* models of CGG-repeat–induced neurodegeneration. Notwithstanding their role(s) in mitigating the phenotypes in animal models, roles for Purα and hnRNP A2/B1 have yet to be demonstrated in human disease or in murine models of FXTAS.	136–138
	Purα	(see above) Transcriptional activator also thought to be involved in the control of DNA replication	(see above)	136–139
	Sam68	Altered mRNA splicing an RNA-binding protein that belongs to the “signal transduction and activation of RNA” (STAR) family	For Sam68, there is clear evidence of insufficiency in animals and humans; with altered splicing noted in FXTAS patients. However, a critical test of these candidate proteins will be whether they have any direct primary or secondary role in FXTAS pathogenesis in humans.	63,65
	DGCR8	With Drosha, reduced processing of microRNA precursors DGCR8, part of the microprocessor complex that processes micro (mi)RNA precursors in the nucleus		136, 140–143
FMRP insufficiency	FMRP			21,71–74
Antisense FMR1 RNAs	RNAs			144–147
RAN translation	polyGly-containing peptides			78,148
R-loop/DNA damage response	R-loops, gH2AX			83,84

Table 2

Findings associated with cerebellar ataxia and/or action tremor in adults[a] that support testing for premutation alleles of *FMR1*

Associated feature	Additional comments	Independent indicator for *FMR1* testing[b]
Family members with intellectual impairment, autism, or autism spectrum disorder (ASD)	Fragile X syndrome is the leading monogenic cause of intellectual impairment and autism/ASD. FXTAS is common among older adults (>50 yr) in fragile X families	Yes
Primary ovarian insufficiency (POI)	Premutation alleles of the *FMR1* gene constitute the leading monogenic cause of POI, often described in terms of early menopause/infertility (<40 yr)	Yes
Hypothyroidism (women)	50% of females with FXTAS, often associated with other evidence of immune dysfunction[7]	No
Peripheral neuropathy	60% of men and 53% of women with FXTAS[149]	No
Muscle pain / (fibromyalgia)	76% / (43%) among women with FXTAS[7]	No
MRI hyperintensities within the middle cerebellar peduncles (MCP) or splenium of the corpus callosum[32,150]	The “MCP sign” was one component of the original definition of FXTAS; involvement of the MCP and/or the splenium is highly-characteristic of FXTAS	Yes
Family member with the premutation	At risk to be a carrier with no symptoms or any premutation- associated disorder	Yes

[a] Typical onset > 50 yr

[b] Features that would warrant testing for CGG-repeat expansions of *FMR1* irrespective of the presence of tremor or ataxia.