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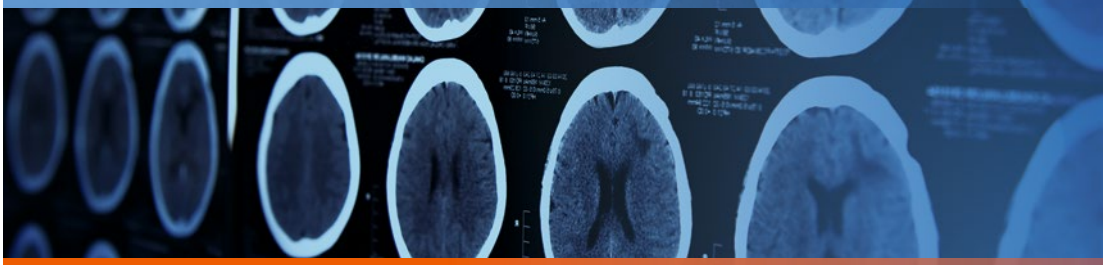
Deborah A. Hall
Editors



FXTAS, FXPOI, and Other Premutation Disorders

Second Edition

Springer

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Chapter 1
Clinical Neurological Phenotype of FXTAS

Maureen A. Leehey, Deborah A. Hall, Ying Liu, and Randi J. Hagerman

Abstract The classic presentation of fragile X-associated tremor/ataxia syndrome (FXTAS) is an aging man with progressive cerebellar gait ataxia, kinetic tremor, mild parkinsonism, cognitive decline, especially executive dysfunction and short-term memory deficiency, and peripheral neuropathy. Autonomic dysfunction and mood/anxiety disorders may be present. MR imaging often reveals global brain atrophy and white matter changes, including hyperintensities of the middle cerebellar peduncles, termed the "MCP sign," and of the splenium of the corpus callosum, and pathology shows intranuclear inclusions, especially in brain. Recent studies, however, have shown that the FXTAS clinical picture is variable, for example, affected persons may have minor or no tremor and others have predominant dementia or peripheral neuropathy. Onset of motor signs in men is typically in the early 60s, and approximately 40 % of carrier men and 8–16 % of carrier women over age 50 develop the disorder. Penetrance is age related, such that 75 % of men ≥80 years of age are affected. While less data exist regarding FXTAS in carrier women, they appear to have similar but less severe motor signs, perhaps less cognitive impairment, and some different patterns of involvement than seen in men. FXTAS, at present, is underdiagnosed largely because the presentation is often a combination of signs which are common in the elderly and because affected persons often lack insight regarding their deficits and are resistant to seeking medical care. Furthermore, the heterogeneous nature of the disorder facilitates misdiagnosis, especially in the earlier stages. Diagnosis requires *FMR1* gene testing. Accurate diagnosis is not only important for the affected person but also for their family, as immediate family members may be at risk of having progeny with fragile X syndrome or other premutation associated problems.

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Introduction

The core clinical features of fragile X-associated tremor/ataxia syndrome (FXTAS) are cerebellar gait ataxia and action tremor (Hagerman et al. 2001; Leehey et al. 2002). Cognitive dysfunction, particularly frontal executive dysfunction, is a very common and disabling manifestation (Grigsby et al. 2008; Juncos et al. 2011) (see Chap. 3). Parkinsonism (Juncos et al. 2011; Niu et al. 2014; Apartis et al. 2012; Hall et al. 2010) and neuropathy (Apartis et al. 2012) can also occur in varying degrees, and other frequent signs are mood/anxiety disorders (Birch et al. 2014; Bourgeois et al. 2011; Adams et al. 2010; Hashimoto et al. 2011a; Seritan et al. 2013a) and autonomic dysfunction (Juncos et al. 2011; Hamlin et al. 2012; Jacquemont et al. 2003). Predictable radiographic abnormalities are global brain atrophy and cerebral and cerebellar white matter changes, including involvement of the middle cerebellar peduncles (Brunberg et al. 2002) (see Chap. 4), the insula, the pons (Hagerman and Hagerman 2013), and the splenium of the corpus callosum (Apartis et al. 2012). Intranuclear inclusions are seen in many areas of the brain in individuals with FXTAS at autopsy (Greco et al. 2002, 2006, 2007) (see Chap. 5) and represent a pathological hallmark of the condition, likely mediated by a RNA toxicity mechanism proposed for this disease (Hagerman and Hagerman 2013; Hagerman et al. 2004) and/or a DNA damage repair mechanism (Hagerman and Hagerman 2015) (see Chap. 6). While these features encompass a "classic" FXTAS presentation (see Fig. 1.1), accumulating data suggest that the clinical phenotype may vary considerably among affected persons. Below is a case report of a subject with a typical presentation of FXTAS.

Case Study

Case 1. A retired geologist was brought to a clinic at age 77 by his wife although he insisted he was not having any problems and did not need to be seen. During the visit he admitted to balance difficulty starting at age 65 when he played tennis. At age 69, his wife noticed bilateral hand tremor, especially when holding up a newspaper. The tremor progressed slowly. The patient still did not appreciate any impairment from shaking nor even acknowledge the tremor. At age 71, he gave up tennis and began having occasional falls. His primary care physician started donepezil, which was not helpful. Antidepressant medication was recommended and refused. At age 74, he took levodopa, but after a year it was stopped as there was no obvious benefit. In recent years people had commented that he stumbled like a "drunken sailor." One year ago he began leaning to the right, and an MRI done for possible

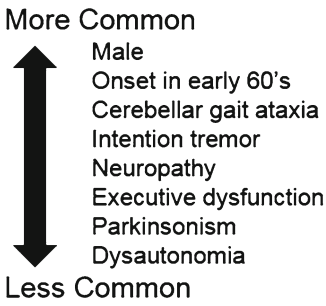


Fig. 1.1 Clinical characteristics of FXTAS

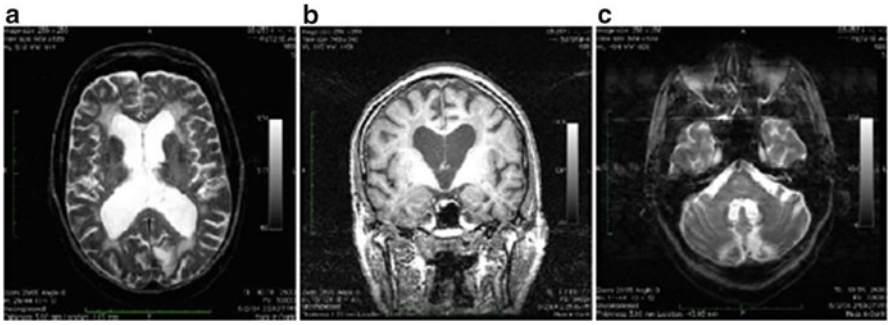


Fig. 1.2 Brain MR images of case 1. A T2-weighted axial image showing large ventricles and cerebral white matter hyperintensities (**a**), a gradient-echo T1-weighted coronal image showing large ventricles and frontal cerebral atrophy (**b**), and a T2-weighted axial image showing there is no hyperintensity in the middle cerebellar peduncles, i.e., the MCP sign (**c**). The latter, when present, is a distinctive finding in FXTAS that occurs in approximately 60 % of affected males and 13 % of affected females (Adams et al. 2007). See Chap. 4 for more details on radiological findings

stroke showed moderate generalized atrophy with very large ventricles and white matter T2 hyperintensities in the cerebral hemispheres and the pons. He was being treated for hypertension and hyperlipidemia and drank two beers per night. There was no history of alcohol abuse. One of his daughters had a son with fragile X syndrome. On examination at age 77, he had a high-average verbal IQ and significantly below-average performance IQ. Short-term memory, capacity to learn and retain new information, and speed of information processing were impaired. MMSE was 26/30. He had slightly masked facies, a mild intention tremor bilaterally in the arms, mild slowing and incoordination of rapid alternating movements, moderately increased tone of a gegenhalten quality in both arms, reduced reflexes in the legs, and no perception of vibration in his great toes. He was unable to perform or even initiate tandem gait. Casual gait was slightly wide based and mildly slow, with irregular foot placement. He leaned to the right and was somewhat stooped forward in a parkinsonian fashion. Repeat MRI showed atrophy and white matter changes (Fig. 1.2), and the radiologist felt it was consistent with normal pressure hydrocephalus. At age 80 he had moderate dementia (MMSE is 21/30), frequent falls, mild intention tremor, and occasional urinary incontinence. Venlafaxine was added for agitation. *FMR1* gene analysis showed 125 CGG repeats.

As an X-linked disorder, FXTAS mainly affects premutation carrier men (Jacquemont et al. 2004), due in part to the protective influence of a second X chromosome in women (Hagerman et al. 2004; Berry-Kravis et al. 2005; Jacquemont et al. 2005). Recently more data has been published regarding women. This data suggests that the premutation may confer different medical risks with aging than those that occur in men, due to hormonal and other, as yet unknown, factors. Chaps. 10 and 12 discuss non-FXTAS phenotypes that occur in carrier women, and a section in this chapter is specifically devoted to the current knowledge regarding carrier women with FXTAS.

The age of onset of one or both of the core motor signs of FXTAS, tremor and ataxia, in men is 61.6 ± 7.9 years (mean ± SD) (Tassone et al. 2007). Using established diagnostic criteria (Jacquemont et al. 2003), approximately 40 % of carrier men and 8–16 % of carrier women over age 50 (Jacquemont et al. 2004; Tassone et al. 2007; Coffey et al. 2008; Rodriguez-Revenga et al. 2009) develop the disorder. Penetrance, however, is age related, such that 75 % of men ≥80 years of age are affected, as shown in Fig. 1.3 (Jacquemont et al. 2004).

There appear to be two dominant phenotypic presentations of classic FXTAS: (a) a tremor-dominant subtype in which the onset of ataxia is delayed; (b) a second in which ataxia is the dominant presentation from the outset. In both subtypes, once ataxia emerges it tends to track frontal cognitive changes ($p < 0.01$) (Juncos et al. 2011).

Clinical Signs of FXTAS

Movement Disorders

The average age of onset of tremor in carrier men is 62.6 ± 8.1 years (range 39–78 years) (Tassone et al. 2007). Most have action tremor as the initial motor manifestation (Juncos et al. 2011; Apartis et al. 2012; Leehey et al. 2007), but in some cases the tremor is minor and not noticed by the patient, as in the case report. It may not be evident until late in disease. In a study of 54 premutation men with a mean age of 67 that were selected without regard to neurological signs (Leehey et al. 2008), 50 % had tremor and the mean severity was rated as slight by movement disorders neurologists. Seventeen percent (9/54) had a tremor in at least one arm that was rated as moderate or severe (unpublished data). In another study of 50 FXTAS men (21 definite, 10 probable and 9 possible, 10 indeterminate FXTAS), 88 % had tremor on neurological examination and 41 % of those were not aware of it. Voice tremor and/or titubation was present in 10 % of patients (Juncos et al. 2011). Head tremor is common. The classic FXTAS tremor is a relatively symmetric kinetic tremor that increases in amplitude at endpoint, and a milder postural tremor is often present. Rest tremor is less common than action tremor, and in at least some of the more severe cases is likely a reemergence of the postural tremor.

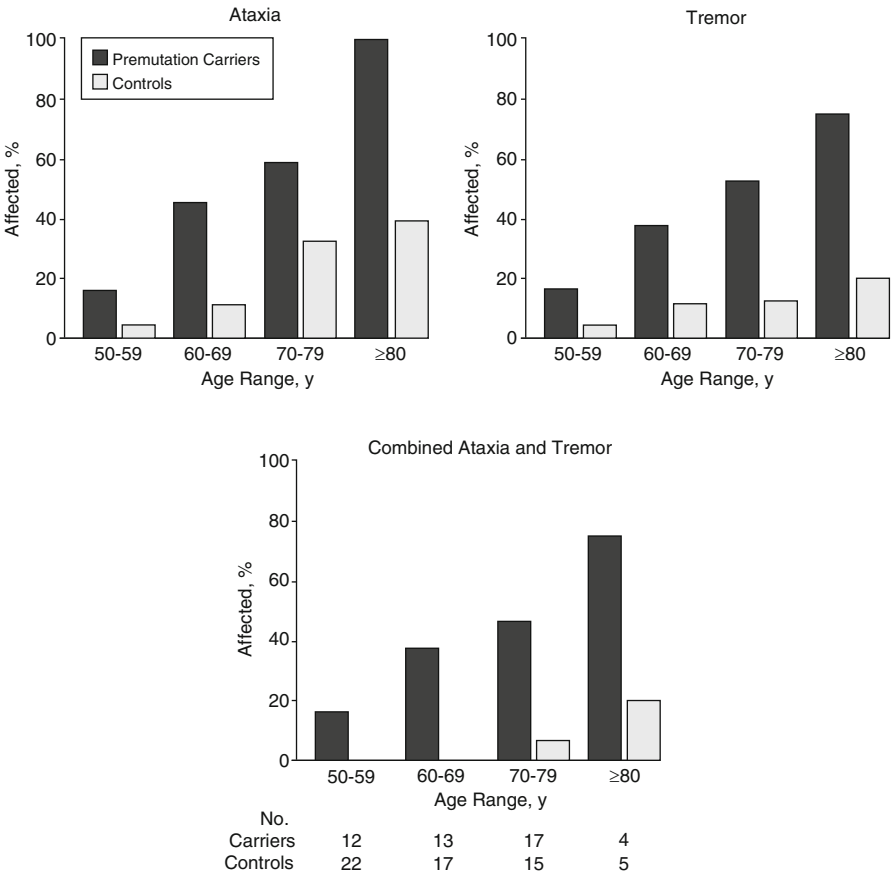


Fig. 1.3 Percentage of men with self-reported symptoms of gait disturbance, intention tremor, or combined ataxia and tremor. Reprinted with permission from Jacquemont et al. (2004); Copyright © 2004, American Medical Association. All Rights reserved

Clinical and electrophysiological characterization of tremor in 18 men and 4 women with definite or probable FXTAS showed three different tremor patterns: essential tremor-like tremor (35 %), cerebellar tremor (29 %), parkinsonian tremor (12 %), and no detectable tremor (24 %) (Apartis et al. 2012). Patients with essential-like tremor had a small amplitude bilateral and symmetric or asymmetric tremor, involving wrist and fingers only. The tremor occurred during postural maintenance and action. Patients with cerebellar tremor had a higher amplitude bilateral proximodistal tremor in the upper limbs. The tremor occurred during postural maintenance and non-goal-directed movements and worsened during target-directed movements. Parkinsonian tremor was characterized by a unilateral upper limb rest tremor. As the disease progresses, the kinetic tremor increases in amplitude and is associated with hypermetria on finger–nose testing. Large-amplitude tremor and dysmetria may interfere greatly with eating, writing, and other daily activities that require fine

motor control. Handwriting becomes large, untidy, and tremulous. Women with FXTAS have a lower frequency of tremor compared to men (Apartis et al. 2012).

The mean age of onset of cerebellar gait ataxia, another defining feature of FXTAS, is 63.6 ± 7.2 years (range 47–78 years) (Tassone et al. 2007). Progressive difficulty with tandem gait (considering normal as 10 perfectly placed consecutive steps) (Huntington Study Group, 1996) likely occurs a few years before the carrier begins falling or develops a feeling of instability. Progressive gait instability is a major source of disability in FXTAS, with falls frequently resulting in injury, such as fractures, lacerations, and even subdural hematomas. Studies using posturography show that individuals with FXTAS have greater postural sway on conditions that test cerebellar and vestibular pathways and suggest deficits in this anatomic Movement disorderspathway and those that serve sensorimotor processing (O'Keefe et al. 2015). In addition, pathology in the corticopontocerebellar loop appears to be responsible for loss of balance control, and abnormalities in the spinocerebellar tracts lead to general balance perturbations in FXTAS patients (O'Keefe et al. 2015). Slowed, poorly coordinated hand movement, i.e., dysdiadochokinesis, and abnormalities of heel–shin testing occur in later stages or in those with a predominately cerebellar presentation. Ataxia, like tremor, sometimes is not noticed by patients (Juncos et al. 2011).

Parkinsonism is another motor feature of FXTAS and has been documented in 29 to 64 % of individuals with FXTAS (Juncos et al. 2011; Niu et al. 2014; Apartis et al. 2012). The major signs are hypomimia and rigidity. Rest tremor is uncommon and is more often seen in persons with advanced disease and prominent action tremor. Some affected persons have a mildly stooped parkinsonian posture. Premutation carriers with parkinsonism had variable Unified Parkinson Disease Rating Scale (UPDRS) scores, ranging from 0 to 50 with a mean score 12.3 ± 12 (Juncos et al. 2011). Generally the parkinsonian signs are mild in FXTAS, with 24 % of premutation carriers initially diagnosed with Parkinson disease (PD) in one study (Hall et al. 2005). These persons generally have an inadequate response to levodopa (Scaglione et al. 2008). However, some carriers have a predominant parkinsonian presentation indistinguishable from primary PD. Hall et al. (2009) described four such premutation carriers, all with good levodopa response.

How the premutation plays a role in parkinsonism in FXTAS has been investigated. [^{123}I]-CIT SPECT (single proton emission computed tomography) imaging has been reported in 14 carriers, ten with FXTAS (seven definite, two probable, one possible; 90 % men) (Scaglione et al. 2008; Zuhlke et al. 2004; Ceravolo et al. 2005; Cellini et al. 2006; Madeo et al. 2013), two with alleles in the gray zone (41 and 51 repeats) and possibly FXTAS (Hall et al. 2010) and two premutation carriers without FXTAS but with cerebellar signs and mild parkinsonism (Hall et al. 2010; Ceravolo et al. 2005). Six of the fourteen had reduced putaminal uptake indicating loss of presynaptic dopaminergic terminals as occurs in idiopathic PD. Three of those six also had iodine-123 iodobenzamide (IBZM) studies of postsynaptic D2 dopamine receptor binding, all showing reduced tracer uptake. Of note, an individual (Kamm

et al. 2005) with 61 repeats whose clinical course was more consistent with multiple system atrophy (MSA) than FXTAS had reduced IBZM uptake, as expected in MSA. While functional imaging results are mixed and larger studies are needed, the current studies suggest that reduced presynaptic dopamine transporter integrity is related to more definite FXTAS diagnosis and higher motor UPDRS scores, thus more advanced disease, and that postsynaptic dopamine striatal activity is also impaired. Some individuals may manifest a complex, mixed disease process. This is supported by the finding of both Lewy bodies and FXTAS intranuclear inclusions on pathological examination of brain from a premutation carrier with a predominantly parkinsonian presentation (Greco et al. 2002).

Cognitive and Psychiatric Signs

Cognitive dysfunction and behavioral changes are a very problematic and disabling aspect of FXTAS (see Chap. 3 for more details). In part, they are likely a source of the common dichotomy between the family's recognition of tremor and other signs and the patient's lack of recognition or concern. Studies suggest that dementia occurs in about 21–50 % of men with the disorder (Juncos et al. 2011; Jacquemont et al. 2004; Bourgeois et al. 2007; Seritan et al. 2008), perhaps less often in women (Hagerman et al. 2004), and that the frequency is higher in late-stage FXTAS. Impairment of cognition in a subset of carriers without motor signs of FXTAS develops as early as middle adulthood, and progressively worsens with increasing age (Grigsby et al. 2008; Cornish et al. 2008, 2011; Hocking et al. 2012). Disturbance of executive functioning, working memory, and information processing are the primary deficits in both genders (Birch et al. 2014; Grigsby et al. 2014; Kraan et al. 2014) (as summarized by Grigsby et al. 2014; Birch et al. 2014; Kraan et al. 2013). It is not possible to localize the cognitive deficits of FXTAS to a specific brain region. Neuropathological studies in FXTAS show extensive involvement of white matter and the cognitive phenotype is consistent with a white matter dementia, in contrast to the impaired cortical function more characteristic of Alzheimer's disease and related disorders. In addition, neurophysiological and other studies implicate frontal lobe impairment, while the prominent executive function deficits along with frequent cerebellar motor signs imply cerebellar involvement (Grigsby et al. 2014).

Psychiatric symptoms are also common (Birch et al. 2014; Bourgeois et al. 2006, 2011; Adams et al. 2010; Hessl et al. 2005; Bacalman et al. 2006; Seritan et al. 2013b) Three studies (Adams et al. 2010; Bacalman et al. 2006; Hashimoto et al. 2011b) compared psychiatric symptoms in FXTAS to those of matched controls. These yielded mixed results. However, reports from the spouse/family members of apathy, irritability, depression, disinhibition, and agitation/aggression were significantly elevated in individuals with FXTAS (Bacalman et al. 2006).

Peripheral Nervous System Findings

The peripheral nervous system is involved in FXTAS and inclusions are common throughout the PNS (Hunsaker et al. 2011). Peripheral neuropathy was present in the five men that were originally described with FXTAS (Hagerman et al. 2001), in 60 % of men in a descriptive study of 20 affected men (Jacquemont et al. 2003), and in 53 % of 17 affected women (Coffey et al. 2008). However, a recent study (Juncos et al. 2011) reported that signs and symptoms of peripheral neuropathy were present in only 20 % of 50 premutation carrier men, the majority of whom had FXTAS. This was not different from age-matched controls (26 %). The frequency of peripheral neuropathy in this study may have been underestimated since it was assessed by patient report and nonquantitative examination. Another recent study found 81 % of 22 persons with FXTAS had peripheral neuropathy (Apartis et al. 2012). A large controlled study (Berry-Kravis et al. 2007) reported that carrier men had more neuropathic signs than controls ($p = 0.0014$) and that the severity of the signs correlated with CGG repeat size and with the presence of ataxia. Carrier women tended to have more neuropathic signs than controls ($p = 0.17$) and severity correlated with ataxia. Neuropathy has been reported as the presentation of FXTAS. A carrier women presented with a painful small fiber neuropathy and was found to have cerebellar signs and MRI abnormalities diagnostic for FXTAS (Chanson et al. 2015). Also, Hagerman et al. (2007) described three men and one woman with FXTAS that presented with neuropathy and a carrier man that had neuropathy and no other signs of FXTAS. Electrophysiological findings in men with FXTAS were found to be consistently abnormal in a controlled (Soontarapornchai et al. 2008) and uncontrolled study (Apartis et al. 2012). Carrier men without FXTAS had slightly but not significantly abnormal testing. CGG repeat length and mRNA level correlated with severity of some of the electrophysiological findings (Soontarapornchai et al. 2008). One study identified different electrophysiologic patterns. Fifty-six percent (9/16) had a non-length dependent sensory neuropathy and 25 % had a length-dependent sensory neuropathy (Apartis et al. 2012). Electrodiagnostic studies document a predominantly axonal sensorimotor polyneuropathy (Soontarapornchai et al. 2008). Usually neuropathic findings are not severe enough to exacerbate ataxia (Juncos et al. 2011; Apartis et al. 2012). Some affected persons have marked neuropathic pain, suggesting involvement of small fibers (Chanson et al. 2015).

Autonomic Symptoms

Autonomic symptoms are common in men with FXTAS, with case series reporting impotence (56–80 %), bowel incontinence (30 %), bladder dysfunction (35–55 %), and orthostatic hypotension (15 %) and hypertension (50–65 %) (Juncos et al. 2011; Apartis et al. 2012; Hamlin et al. 2012; Jacquemont et al. 2003). Erectile dysfunction typically begins before the onset of tremor and ataxia and bladder and bowel incontinence occur late in the course of FXTAS (Greco et al. 2007). A controlled study found that carrier men reported urinary incontinence more than controls, but

the difference was not significant ($p=0.07$) and there was no difference in women (Jacquemont et al. 2004). Another study compared scores on the Scales for Outcomes in PD-Autonomic (SCOPA-AUT) rating scale in 56 premutation men to PD, Huntington disease, and published controls. Carriers reported more urinary symptoms than published controls ($P<0.01$), but had no difference in other autonomic symptoms. The urinary symptoms were significantly correlated with tremor, ataxia, and Montreal Cognitive Assessment scores, indicating worsening with increased disease severity. Interestingly, the carriers had higher SCOPA-AUT scores than the Huntington disease subjects but lower than the PD subjects. Urinary and fecal incontinence is present in late-stage FXTAS (Greco et al. 2007; Leehey et al. 2007; Gokden et al. 2009). Autonomic dysfunction is thought to be a consequence of involvement of the peripheral nervous system (Hunsaker et al. 2011; Gokden et al. 2009). There are case reports of carriers with symptomatic orthostasis or syncope (Kamm et al. 2005; Gokden et al. 2009; Louis et al. 2006; Pugliese et al. 2004). Pugliese and colleagues (2004) reported a 73-year-old man that presented with postprandial hypotension and was found to have had bilateral hand tremor for two years and 73 CGG repeats in his *FMR1* gene. The patient had no ataxia or family history suggestive of *FMR1*-related disorders. Louis et al. also reported a 77-year-old man who met clinical and autopsy criteria for FXTAS who had episodes of syncope an hour after a meal or during a large bowel movement (Louis et al. 2006). Syncope should include a workup for bradycardia or arrhythmias since the cardiac conduction system can be involved with inclusions (Hunsaker et al. 2011; Gokden et al. 2009). Some men with FXTAS have required placement of a pacemaker. Involvement of the autonomic nervous system has been documented in neuropathological studies which showed intranuclear inclusion bodies typical of FXTAS in paraspinal sympathetic ganglia (Greco et al. 2006), myenteric ganglia of the stomach, and subepicardial autonomic ganglia (Gokden et al. 2009).

Controlled studies find that hypertension is more common in carriers with FXTAS. In a cohort of men, including 92 premutation carriers without FXTAS, 100 with FXTAS, and 183 controls, a significantly elevated odds ratio (OR) of hypertension relative to controls for premutation carriers with FXTAS (OR = 3.22, 95 % CI: 1.72–6.04; $P=0.0003$) was found among participants over 40 years old. The age-adjusted estimated odds of hypertension in premutation carriers without FXTAS in the over 40-year-old age group was higher compared to controls (OR = 1.61, 95 % CI: 0.82–3.16), but was not statistically significant ($P=0.164$) (Hamlin et al. 2012). Women with FXTAS also have hypertension more than matched controls ($p=0.002$) (Coffey et al. 2008). Further, anecdotal evidence suggests that cardiac dysfunction, e.g., congestive heart failure, may occur more frequently in men with FXTAS in the later stages, but controlled studies are needed.

Other Clinical Findings

Other disorders appear to be more common in premutation carriers and/or FXTAS. Among 50 premutation carriers, most of whom had FXTAS, 50 % reported hearing loss ($p=0.002$). Ninety-eight percent of them had previously documented

sensorineural hearing loss (Juncos et al. 2011). In the same population 20 % had spouses that reported nighttime activity suggestive of REM sleep behavior disorder; this reached significance ($p < 0.0001$) compared to a historical control. The prevalence of migraine was 54.2 % in carrier women and 26.79 % in carrier men compared to matched female, 25.3 % ($p = 0.0001$), and male controls, 15.5 % ($p = 0.041$) (Au et al. 2013). Olfactory identification capacity was measured in 41 premutation carriers and 42 controls using the University of Pennsylvania Smell Identification Test. Frequency of olfactory defects was higher in carriers compared to controls (61 % vs. 29 %, $P = 0.003$) (Juncos et al. 2012). Premutation carriers with and without FXTAS reported symptoms of restless legs syndrome 1.9 times more than controls and those affected endorsed worse symptoms than controls (Summers et al. 2014). Further, a questionnaire study suggested that persons with FXTAS have a 3.4-fold risk for sleep apnea compared to matched controls; the authors hypothesized that significant hypoxia at night may worsen FXTAS (Hamlin et al. 2011).

Eye movement abnormalities in FXTAS have been reported (Scaglione et al. 2008; Sulkowski and Kaufman 2008; Fraint et al. 2014). Three of five men with FXTAS had a vertical gaze palsy (Scaglione et al. 2008). Fraint et al. (2014) detailed eye findings in 19 persons with FXTAS, and found five had a PSP-like phenotype, including decreased optokinetic nystagmus, especially in the vertical direction, slowed vertical saccades, and square wave jerks. One of his cases, a 75-year-old woman with FXTAS had absent vertical optokinetic nystagmus, slowed vertical saccades, and square wave jerks. In this cohort other frequent findings were saccadic pursuits, lateral end gaze nystagmus, dysmetric saccades, and slowed saccades. Another study reported an 80-year man with FXTAS who had acquired diplopia, strabismus, and other oculomotor abnormalities (Sulkowski and Kaufman 2008).

Although not a clinical study of FXTAS, Seltzer et al. (2012) reported the prevalence of symptoms that occur in FXTAS in their population-based sample of 30 premutation carriers who were in their mid-60s. Compared with controls, there was a significantly higher rate of dizziness/faintness (17.9 % weekly or more often for the premutation group vs. 3.9 % for the controls, $P < 0.001$) and numbness (28.6 % weekly or more often for the premutation group vs. 13.3 % for the controls, $P < 0.05$). There have been two case reports of monoclonal gammopathy of unknown significance (Fraint et al. 2014; Sumekar et al. 2011), one of generalized cortical reflex myoclonus (Poston et al. 2010) and one of pathological crying (Van Ballaer and Vandenbulcke 2014) in FXTAS. In late-stage FXTAS, problems with swallowing, sedation in the day, and muscle weakness are common (Hagerman and Hagerman 2013; Leehey et al. 2007).

Premutation Carrier Women

Premutation carrier women have unique medical concerns not reported in men. For example they have a higher rate of primary ovarian insufficiency (POI), approximately 18 % (Coffey et al. 2008; Rodriguez-Revenga et al. 2009), compared to other

women (Cronister et al. 1991). Fragile X-associated POI (FXPOI) is discussed in Chap. 10. Having FXPOI, however, does not apparently increase the risk of FXTAS in women (Coffey et al. 2008), although prospective studies are needed.

Besides FXPOI, disorders found more often in women when collecting historical information compared to men with FXTAS include autoimmune thyroid disorders, chronic muscle pain, fibromyalgia and, as previously discussed, migraine headaches (Coffey et al. 2008; Rodriguez-Revenga et al. 2009; Au et al. 2013; Winarni et al. 2012). Approximately 50 % of women with FXTAS have thyroid dysfunction, usually hypothyroidism (Coffey et al. 2008; Winarni et al. 2012). This problem may be diagnosed in early or mid-adulthood, usually long before neurological difficulties. Chronic muscle pain is reported in 24 % (Rodriguez-Revenga et al. 2009) and 35 % (Coffey et al. 2008) of women with FXTAS, compared to 10.7 % in controls (Coffey et al. 2008; Winarni et al. 2012). Likewise, fibromyalgia is reported in 25 % (Winarni et al. 2012) and 43.8 % (Coffey et al. 2008) of women with FXTAS compared to 4.2 % (Winarni et al. 2012) and 9.4 % (Coffey et al. 2008) in controls.

Daughters of men with FXTAS are more likely to report balance problems and neurological symptoms than daughters of premutation carrier fathers without FXTAS (Chonchaiya et al. 2010). Sleep problems were the most common symptom reported for these women and this problem may be related to their anxiety and chronic worries which can be disruptive of sleep at night. (Chonchaiya et al. 2010; Besterman et al. 2014) In addition, chronic pain such as neuropathy or fibromyalgia can lead to sleep problems and exacerbate psychological problems (Jalnapurkar et al. 2015). The use of opioids or other substance abuse to treat chronic pain has the potential to exacerbate the progression of white matter disease and FXTAS, as has been reported in a few case studies (Muzar et al. 2014, 2015).

Cognitive decline has been found to be less frequent in women compared to men with FXTAS (Seritan et al. 2008). However, carrier women without FXTAS have visuospatial deficits (Goodrich-Hunsaker et al. 2011), attention problems (Hunter et al. 2008), and language dysfluencies that progress with age (Sterling et al. 2013). Women with FXTAS demonstrated lower performance in verbal learning and executive function and a significant reduction of the N400 congruity effect in ERP analyses (Yang et al. 2014). Karmon and Gadoth (2008) reported that a 62-year-old women with 75 repeats had classic FXTAS including severe dementia. In a clinical-neuropathological cases series of eight autopsied premutation carrier women, four had dementia; of the four, three had FXTAS diagnosed before death. Postmortem examination revealed the presence of intranuclear inclusions in all eight cases. Among the four subjects with dementia, three also had neuropathological findings of Alzheimer's disease in addition to their FXTAS (Tassone et al. 2012). Thus dementia in FXTAS occurs from FXTAS alone but may also be from or potentiated by coexistent Alzheimer's disease.

The most common problems of women with the premutation in mid-adulthood are anxiety and depression (Franke et al. 1996, 1998; Sobesky et al. 1996; Roberts et al. 2009). This is addressed in Chap. 12.

The following case history demonstrates how a woman with FXTAS may present differently than an affected man.

Case Study

Case 2. A 79-year-old woman presented with weakness and inability to walk. Her neurological symptoms began at age 67 with pain in her legs and a feeling of tightness in her feet as if she was wearing tight boots. She also developed pain in the muscles of her low back. She was treated with hydrocodone and required higher doses as the pain became chronic. She developed hypertension and swelling in her legs. Electrodiagnostic studies documented a length-dependent polyneuropathy. At age 75, she was diagnosed with fibromyalgia by her rheumatologist and chronic inflammatory demyelinating polyneuropathy by her neurologist. She was treated with intravenous gamma globulin and prednisone but her symptoms worsened. By age 76, she needed a four-pronged cane due to weakness in her legs and gait ataxia; 2 years later she was dependent on a wheelchair. Her family noticed an intention tremor at age 78, but the patient denied any tremor. When examined at age 79, she had marked weakness and severe edema in the lower extremities with absent ankle reflexes. She had no vibration sense and decreased cold and pinprick sensation in her distal lower extremities. She was unable to walk or stand, even with full support. An action tremor was present in the right hand. Cognition was normal for age but she appeared depressed. Brain MRI demonstrated moderate brain atrophy and widespread subcortical and periventricular white matter disease without a clear MCP sign. *FMR1* gene analysis showed her CGG repeat sizes to be 30 and 73. She has ten children, many of whom are carriers, and multiple grandchildren with fragile X syndrome.

Natural History of FXTAS

Formal study of the natural history of FXTAS consists of only one retrospective chart review study (Leehey et al. 2007). This report studied the progression of tremor and ataxia in 55 men with FXTAS. After the initial motor sign, usually tremor, median delay of onset of ataxia was two years, onset of falls six years, dependence on a walking aid 15 years, inability to do most daily activities 16 years, and death 21 years. However, life expectancy ranged from 5 to 25 years, and several cases of death within 5–7 years after seeking medical care have been reported (Greco et al. 2006; Kamm et al. 2005; Gokden et al. 2009; Louis et al. 2006). Cause of death is usually due to cardiopulmonary problems, including pneumonia often secondary to aspiration, cardiac arrest, congestive heart failure, and progression of neurological disease. In the months before death, patients are bedridden, dysarthric, dysphagic, without bladder or bowel control, rigid, and bradykinetic (Greco et al. 2007; Leehey et al. 2007; Louis et al. 2006).

Diagnosis of FXTAS

While tremor and ataxia are the core motor signs in FXTAS, accumulating literature documents the wide variation in clinical presentation. Some persons present with predominant dementia (Bourgeois et al. 2006; Goncalves et al. 2007), neuropathy (Chanson et al. 2015; Hagerman et al. 2007), parkinsonism (Hall et al. 2010; Louis et al. 2006), dysautonomia (Pugliese et al. 2004), or action tremor without much ataxia (Leehey et al. 2003). Some have spasticity in the legs and other pyramidal tract signs (Jacquemont et al. 2005; Cellini et al. 2006; Kamm et al. 2005). A retrospective study of diagnoses given to persons with FXTAS ($n=56$) before FXTAS was a known clinical entity demonstrates the heterogeneity of presentations: 24 % were diagnosed with parkinsonism, 20 % with tremor, 17 % with ataxia, 13 % with dementia, 10 % with cerebrovascular disease, and 16 % with other miscellaneous disorders (see Chap. 2). The latter included multiple sclerosis, benign positional vertigo, peripheral neuropathy, and normal pressure hydrocephalus (Hall et al. 2005). Moreover, affected persons within the same family may present with different neurological features (Peters et al. 2006; Capelli et al. 2007). The heterogeneity in clinical signs is probably due to the widespread pathological involvement of both the central and the peripheral nervous systems. In addition, some of this variability is likely due to the size of the CGG repeat, since age of onset (Tassone et al. 2007) and death (Greco et al. 2006) correlate negatively and the severity of motor signs (Leehey et al. 2008) and degree of brain atrophy (Loesch et al. 2005) correlate positively with repeat size.

The protean presentation of FXTAS makes diagnosis difficult and the differential diagnosis broad. Other disorders that can have presentations similar to FXTAS are listed in Table 1.1. The diagnosis of multiple system atrophy is made when established diagnostic consensus criteria (Gilman et al. 2008) of autonomic, parkinsonian, and cerebellar dysfunction are met. In one study (Kamm et al. 2005), less than 1 % of persons meeting previously established but still stringent diagnostic criteria (Gilman et al. 1999) had FXTAS, but approximately 4 % of persons with the cerebellar subtype had FXTAS. The presence of unusual features in a person meeting diagnostic criteria for multiple system atrophy, e.g., a prolonged course or a prominent tremor, should prompt *FMR1* gene testing (Kamm et al. 2005). Another area of FXTAS diagnostic overlap is late-onset cerebellar ataxia. Studies screening populations with cerebellar ataxia report that approximately 2 % of cases were *FMR1* carriers (see Chap. 2) (Jacquemont et al. 2006). This frequency is comparable to the frequency of any of the individual spinocerebellar ataxias, and yield would be increased if the patient is male and has onset over age 50. Ataxia that is assumed to be from strokes or cervical spine stenosis in an older male may instead be from FXTAS (Hall et al. 2005), particularly if there are concomitant signs consistent with FXTAS. A further area of FXTAS diagnostic overlap is dementia. Alzheimer's disease can be distinguished from FXTAS because persons with Alzheimer's have deficits in encoded memory and language, while persons with FXTAS have relative

preservation of these but have deficits in retrieval and executive function (Seritan et al. 2008). White matter MRI changes, mild parkinsonism, and dementia occur in both vascular dementia and FXTAS, but these two disorders may be distinguished because of the prominent executive dysfunction in FXTAS and other concomitant FXTAS signs.

Diagnostic Criteria for FXTAS

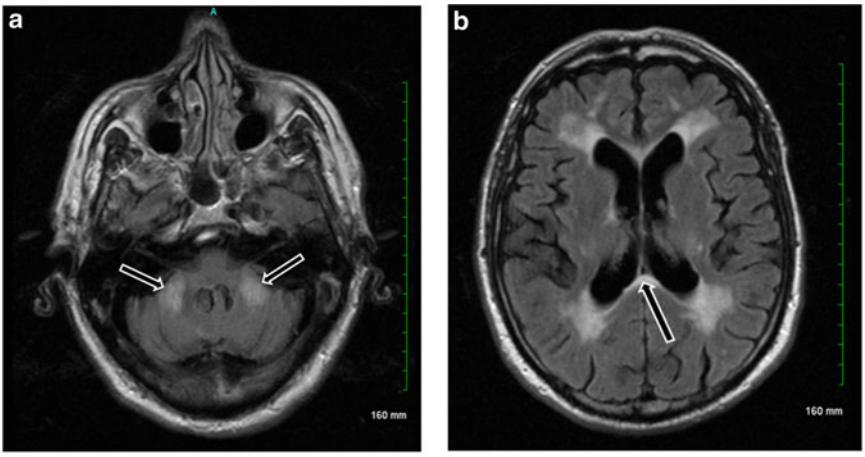
When FXTAS is suspected, diagnostic evaluation requires *FMR1* gene testing, and MR imaging is useful in documenting the degree and type of brain involvement. Additional studies may be warranted when patients have specific signs, such as testing for reversible causes of dementia and electrodiagnostic studies to characterize neuropathy. Diagnostic criteria for FXTAS were established in 2003 (Fig. 1.4) (Jacquemont et al. 2003; Hagerman and Hagerman 2004) and later revised (Hall et al. 2014). The initial description of FXTAS consisted of a neurodegenerative disorder in premutation carriers, mostly in men over age 50, characterized by intention tremor, cerebellar gait ataxia, and parkinsonism, as well as brain atrophy and often middle cerebellar peduncle hyperintensities (the "MCP sign") on magnetic resonance

Table 1.1 Differential diagnosis of FXTAS

Multiple system atrophy, especially the cerebellar subtype
Late-onset cerebellar ataxia
Essential tremor
Parkinsonism, especially atypical presentations
Spinocerebellar ataxia[a]
Secondary ataxia
Alzheimer's disease
Peripheral neuropathy[b]
Vascular dementia
Multiple sclerosis
Normal pressure hydrocephalus
Progressive supranuclear palsy (PSP)
Frontotemporal dementia
Alcoholic tremor/ataxia

[a]Spinocerebellar ataxia (SCA) type 12 is particularly similar to FXTAS since it is characterized by cerebellar gait ataxia, action tremor, and dementia. However, onset of SCA 12 is usually in the fourth decade

[b]The hereditary neuropathies, Charcot–Marie–Tooth disease, should also be considered in the differential diagnosis of FXTAS



c

Diagnostic Criteria		
Molecular	Required	*FMR1* mutation including gray zone
Clinical	Major	Intention Tremor
	Major	Cerebellar gait ataxia
	Minor	Parkinsonism
	Minor	Neuropathy
	Minor	≥ Moderate short term memory deficit
	Minor	Executive function deficit
Radiological	Major	MRI white matter lesions in MCPs or brainstem
	Minor	MRI white matter lesions in the splenium of the corpus callosum
	Minor	MRI cerebral white matter lesions
	Minor	≥ Moderate generalized brain atrophy
Neuropathology	Major	Ubiquitin-positive intranuclear inclusions
Diagnostic Categories		
Definite	One clinical major + [one radiological or pathological major]	
Probable	Two clinical major or [one clinical minor + one radiological minor]	
Possible	One clinical major + one clinical minor	

Fig. 1.4 Diagnostic criteria for FXTAS. Radiological criteria are shown on axial flair T2-weighted MR images, bilateral hyperintensities of the middle cerebellar peduncles (**a**) and hyperintensities of the splenium of the corpus callosum (**b**). The diagnostic criteria required for each diagnostic category is listed in (**c**). The molecular criterion has been amended to allow for the diagnosis in individuals carrying gray zone or full mutation with a lack of methylation or mosaic alleles

imaging (MRI) scans (Hagerman et al. 2001; Leehey et al. 2002; Apartis et al. 2012; Jacquemont et al. 2003). Diagnostic criteria (Jacquemont et al. 2003; Hagerman and Hagerman 2004) were proposed based on this, with the addition of the neuropathological hallmark, intranuclear inclusion bodies (Hagerman and Hagerman 2004), soon after. There has been an enormous amount of literature suggesting that the disorder has additional features (Hall et al. 2014), including peripheral neuropathy and MRI T2 hyperintensities in the splenium of the corpus callosum (CCS) (Apartis et al.

2012). Neuropathy is common enough to be a minor clinical diagnostic criterion, but too nonspecific and common in the aging population to be classified as a major criterion (Hall et al. 2014). CCS hyperintensities were as frequent as MCP hyperintensities, and were useful in identifying patients who had no MCP sign (Apartis et al. 2012). Thus, Hall et al. (2014) proposed these be added as a major criterion for FXTAS. However, the CCS hyperintensities are not unusual in the aging population so here, in Fig. 1.4, it has been added as a minor MRI diagnostic criterion.

FXTAS was initially described in *FMR1* premutation carriers. Recent reports, however, have shown that some individuals carrying a gray zone expansion (41–54 *FMR1* CGG repeats) have a classic FXTAS picture (Hall et al. 2012; Liu et al. 2013). The reason that gray zone carriers may develop clinical disorders is because molecular changes in some gray zone carriers occur similar to that seen in the premutation. As the repeat size increases from 39, the upper range considered normal, there is an increase in the level of *FMR1* mRNA and a decrease in *FMR1* protein (Loesch et al. 2007; Kenneson et al. 2001). Classic FXTAS has also been reported in persons with an unmethylated full mutation or a mosaic full (Loesch et al. 2012; Pretto et al. 2013; Santa Maria et al. 2014). These cases had elevated *FMR1*-mRNA even though they had a full mutation so RNA toxicity can develop in these cases. These individuals would meet diagnostic criteria except that they were not premutation carriers. Given these findings, the diagnostic criteria for FXTAS has been amended to allow for the diagnosis in individuals carrying gray zone or full mutation with a lack of methylation or mosaic alleles (Hagerman and Hagerman 2015; Hall et al. 2014).

Guidelines for Testing

Before *FMR1* gene testing, genetic counseling for the patient and concerned family members is essential (McConkie-Rosell et al. 2007) (see Chap. 14). Some elderly symptomatic persons are reluctant to undergo genetic testing since disease modifying therapy is not yet available. However, treatment is available for symptoms and accurate diagnosis is important for the patient. Diagnosis is also very important for the family, as immediate family members may be at risk of having progeny with fragile X syndrome or a premutation associated disorder.

Guidelines for deciding whom to test have been proposed and are presented in Table 1.2. In general, a reasonable way to keep the diagnosis of FXTAS in mind when seeing patients with any of the many clinical signs consistent with FXTAS is to include questions about *FMR1*-related disease when obtaining family history. One should ask about fragile X syndrome (developmental delay, learning disability, autism), POI, psychiatric symptoms, chronic muscle pain, and FXTAS. Thus, the history of a grandchild with a form of developmental delay should immediately alert the clinician to consider FXTAS.

The presented guidelines (Table 1.2) are for diagnosis of symptomatic persons, but persons without symptoms who are at risk for the carrier status should also

Table 1.2 Testing guidelines for FXTAS

Cerebellar ataxia of unknown cause, onset ≥50 years old
Action tremor of unknown cause, onset ≥50 years old, if also has cerebellar ataxia, parkinsonism, or dementia
Dementia of unknown cause, onset ≥50 years old, if also has cerebellar ataxia, parkinsonism, or action tremor
Multiple system atrophy, cerebellar subtype
Some FXTAS signs
MRI hyperintensities within MCP or splenium of the corpus callosum
Patient history or family history of premature ovarian insufficiency
Family history of an *FMR1* disorder

MCP sign, increased T2 signal intensity in the middle cerebellar peduncles

consider *FMR1* testing. A positive result would enable them and their family to understand important health risks. The presence of MRI hyperintensities within MCP, family history of *FMR1* mutation that confers at-risk premutation carrier status, and patient history of POI, even without signs of FXTAS, are appropriate criteria for *asymptomatic* testing.

For a number of reasons, FXTAS at present is probably underdiagnosed. First, since the initial report was only published in 2001, many physicians are not yet familiar with the disorder. Further, the types of doctors seeing these patients are not the ones that are currently most aware of it. A retrospective chart review study showed that 70 % of persons diagnosed with FXTAS were being managed by general neurologists, 26 % by primary care physicians, and only 4 % by movement disorders neurologists (Hall et al. 2005). Movement disorders neurologists, the physicians that are most aware of FXTAS at present, generally treat persons with ataxia and tremor. But persons with FXTAS are not being referred to movement disorders neurologists because their presentation is often a combination of signs, e.g., tremor, ataxia, and dementia, which are common in the elderly. The latter is another reason that the diagnosis is missed—the nonspecific presentation with signs common in the elderly. Individually, these signs could be due to a variety of causes in the elderly and thus, even when they present in combination, their etiology is often not pursued. Finally, the disorder is hard to recognize in some cases because the heterogeneous nature of the disorder facilitates misdiagnosis, especially in the earlier stages.

Summary and Future Perspectives

FXTAS is the most common single gene cause of tremor and ataxia, but at present the diagnosis is often missed. To improve the frequency of diagnosis, neurologists and primary care physicians need to be educated about the disorder and a family history of *FMR1*-related signs, especially any form of developmental delay, should be obtained in suspect patients. Physicians need to be aware of the heterogeneity of

the clinical presentation and that the patient may simply present with a combination of signs that are common in the elderly. While the MCP sign on MR imaging is helpful in recognizing the disorder, the sign is present in only ~60% of affected persons. Thus, a negative finding does not rule out FXTAS. Guidelines for which clinic patients are most appropriate for gene testing have been presented (Table 1.2). Accurate diagnosis is essential, not only for the patient but also for the family, so they can be informed of important genetic and health risks. Before *FMR1* gene testing, patients and families need genetic counseling.

Most of the research on FXTAS has been done in men. Studies to date find that women are less commonly affected and that they have similar but less severe motor signs. Interestingly, the frequency of muscle pain/fibromyalgia and thyroid disease is higher in women with FXTAS than controls, and this has not been found in affected men. Prospective studies in carrier women are needed to define their full phenotype.

Further research is needed in other areas also. Genetic, epigenetic, and environmental factors that predispose carriers to develop FXTAS need identification. A prospective study of disease progression and modifying factors would provide valuable information needed for life planning. Prospective data and validated reliable quantitative tools are needed for measurement of disease in cross-sectional, longitudinal, and therapeutic trials. To date only one randomized, controlled trial to study modification of progression of FXTAS has been conducted (Seritan et al. 2014). Work in this area is an important direction for future studies.

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Chapter 2
The Epidemiology of FXTAS

Deborah A. Hall and Marsha Mailick

Abstract Recent epidemiological studies in the United States estimate the prevalence of the *FMR1* premutation as ranging from 1/148 to 1/209 in women and from 1/290 to 1/468 in men, with greater variability in prevalence reported in studies internationally. Population studies investigating the prevalence of FXTAS in the general population have not been conducted due to the rarity of the disorder. The prevalence of FXTAS is estimated to be 1/4000 in men over the age of 55, due to age-dependent penetrance. The prevalence in women is thought to be much lower, at approximately 1/7800, because of the protection of the second X chromosome. Many screening studies have been conducted in movement disorder populations, attempting to ascertain undiagnosed FXTAS cases and premutation expansions. These studies have yielded low rates, with a rate of 1 % in cerebellar ataxia patients, <1 % in parkinsonian disorders, such as Parkinson disease and multiple system atrophy, and 0 % in essential tremor. Screening studies vary widely in the type of patients included, both in ethnicity and in gender. Wider inclusion criteria for screening could increase the rates of ascertainment of both FXTAS and premutation expansions in future studies.

Keywords *FMR1* • Epidemiology • Premutation • FXTAS • Prevalence

Introduction

Population-based studies to determine the prevalence of FXTAS have not been conducted due to the estimated low frequency of affected individuals. The current estimate of 1/4000 males over the age of 50 having FXTAS was obtained through an indirect approach of combining the known prevalence of the premutation in the general population and data on penetrance of FXTAS in premutation carriers (Dombrowski et al. 2002; Jacquemont et al. 2004; Rousseau et al. 1995). This estimate, however, conflicts with studies which have found low rates of premutation

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alleles in various movement disorder populations. The prevalence rate of premutation alleles has been studied in both the general population and selected neurological populations. Other epidemiological features of the premutation expansion, such as incidence or mortality ratios, have not yet been studied or defined. Population studies investigating *FMR1* expansions were initiated after discovery of the gene mutation for fragile X syndrome. The methods of quantifying premutation range expansions have improved over time as laboratories have gained experience in the technique, with some of the earlier studies having difficulty with amplification. Problems with methods will be noted throughout the chapter and their likely ramification on estimations of prevalence.

Patients with FXTAS have previously been given diagnoses of tremor (20 %), ataxia (17 %), or parkinsonism (24 %) by their treating physicians (Hall et al. 2005). This has led to the screening of various movement disorder populations for a *FMR1* repeat expansion in the premutation range. Two papers that reviewed this topic in detail were published in 2006 (Hall et al. 2006; Jacquemont et al. 2006). Adult neurological populations not specifically ascertained for a particular movement disorder have not been screened in detail because it has not been considered common for individuals with FXTAS to receive prior diagnoses of a neurological disorder other than a movement disorder. This chapter will review the prevalence of the premutation allele in the general population and in movement disorder populations. Combining these data with the penetrance studies on FXTAS, we will provide estimates for the prevalence of this neurodegenerative disorder in men and women.

Prevalence of the Premutation in the General Population

Several studies have estimated the prevalence of the premutation in the general population. We have summarized the larger and more recent studies in Table 2.1. Many of the earlier and smaller studies used different allele sizes as the lower boundary for defining the premutation allele.

Women

A total of 92,997 women have been screened in the general population. These women were not selected for a family history of intellectual disability. These studies fall into two categories: those performed in a clinical setting and those performed as research. The 60,477 women screened in Israel represent data collected preconceptionally or prenatally in clinic and analyses were performed in a diagnostic laboratory. This is due to the fact that in Israel, screening is provided to all women of reproductive age on a self-pay basis. In these reports, prevalence rates range from 1/116 to 1/159 (Berkenstadt et al. 2007; Geva et al. 2000; Toledano-Alhadef et al. 2001). The ethnic background of the population screened in Israel is very diverse (65 % European, 20 % Middle Eastern and Persian, 15 % North African), and no

Table 2.1 Population studies

Study	Population size	Sex	Number of premutation alleles (CGG)							Prevalence rate
			55–60	61–65	66–70	71–75	76–80	81–200	Total	
Dombrowski	10,572	M	4	3	1	0	1	2	10	1/813
Tzeng	10,046	M	2	1	1	0	1	1	6	1/1674
Rife	5000	M	3	0	0	0	1	0	4	1/1250
Fernandez-Carvajal	5267	M	13	3	4	0	1	0	21	1/251
Rousseau	10,624	F	11	10	6	6	3	3	39	1/272
Geva	9660	F	na	na	na	na	na	na	61	1/159
Toledano	14,334	F	62	15	25	4	9	9	124	1/116
Pesso	9459	F	40	9	5	0	2	6	61	1/153
Berkenstadt	36,483	F	na	na	na	na	na	na	231	1/158
Tassone	14,207	M,F	23	9	3	5	2	8	50	1/209F, 1/430M
Seltzer	6e747	M/F (total)	2/12 (14)	3/3 (6)	1/2 (3)	0/1 (1)	1/0 (1)	0/5 (5)	7/23 (30)	1/151F, 1/468M
Maenner	19,991	M/F (total)	13/33 (46)	4/11 (15)	4/4 (8)	2/6 (8)	1/3 (4)	2/15 (17)	26/72 (98)	1/148F, 1/290M

Cases with a family history of mental retardation are excluded
na data not communicated
[a]Pesso et al. present a preliminary report of the larger data set reported by Berkenstadt et al.

differences in prevalence rates were noted between these groups. A large study in Canada screened 10,624 women and was performed on leftover hematology samples which were pooled for the analysis (five samples per analysis). The reported prevalence rate 1/259 (95 % confidence interval: 1/198–1/373) was significantly lower than the rate reported in Israel. This may be due to an identified French founder effect or to the pooling method, which does not have a sensitivity of 100 % (Rousseau et al. 1995). A third screening study performed in a Caucasian population using diagnostic procedures was performed by Ryynanen et al. (1999) and found a prevalence of 1/246 using a cutoff of 60 CGG repeats (not shown in Table 2.1). Although, the author does not provide the data on alleles between 55 and 60, data from other studies (Table 2.1) show that moving the cutoff from 60 to 55 CGG repeats roughly doubles the prevalence rate, which would then be similar to those published in the Israeli populations. It has also been suggested that the higher premutation frequency in the Israeli studies may be the result of self-referral in the case of a family history of mental retardation. To correct for this possible bias, Berkenstadt et al. excluded 3596 out of 40,079 women who had such a family history. In the latter group, the prevalence was slightly higher at 1/128 (Berkenstadt et al. 2007).

Three recent studies provide US population-based estimates. DNA samples were screened from 3474 Caucasian women drawn from the general population, finding a premutation prevalence rate of 1 in 151 (95 % confidence interval: 1/105–1/249) (Seltzer et al. 2012). A second study screened DNA from a population biobank consisting of 11,527 Caucasian women and reported a similar premutation prevalence

rate of 1 in 148 women (95% confidence interval: 1/113–1/207) (Maenner et al. 2013). A third screening study of a more ethnically and racially diverse study was reported in which 6±895 female samples were screened and a prevalence rate of 1 in 209 women was reported (95% confidence interval: 1/149–1/303) (Tassone et al. 2012). The prevalence of premutation varied by ethnicity; 1 in 123 Asian samples, 1 in 201 Caucasian samples, 1 in 168 Black samples, 1 in 570 Hispanic samples were found to have a premutation (Tassone et al. 2012).

Men

The studies performed in males represent much smaller groups with a total of 49,939 individuals screened (Table 2.1). The variation of prevalence rates (from 1/251 to 1/1674) is much larger than what is seen in the female studies. Ethnic background likely accounts for some of the discrepancies, with very low rates found in the Asian population. On the other hand, two studies performed in Spain (Fernandez-Carvajal et al. 2009; Rife et al. 2003) using the same ascertainment method in both studies (neonatal blood spots) found extremely different rates (1/1250 and 1/251, respectively), so there are clear sensitivity issues in some studies. False positives seem less likely, since all positive tests are replicated. A similar study with neonatal blood spots in a very small sample of 1459 males yielded a rate of 1/730 (two premutation carriers identified) (Saul et al. 2008).

The three recent US prevalence studies yielded relatively high rates. In Seltzer et al. (2012), the Caucasian prevalence rate in men was 1 in 468 (95% confidence interval: 1 in 251 to 1 in 1628). In Maenner et al. (2013), the Caucasian prevalence rate in men was even higher—1 in 290 men (95% confidence interval: 1 in 194 to 1 in 530). In the ethnically and racially diverse screened population reported in Tassone et al. (2012), the prevalence rate for male samples was 1 in 430 (95% confidence interval: 1/268–1/736). As with the female samples they screened, the prevalence of the premutation in males varied by ethnicity; 1 in 358 in Caucasian samples, 1 in 428 for Asian samples, 1 in 595 for Hispanic samples, and 1 in 780 Black samples were found to have a premutation in the Tassone et al. study.

In summary, despite numerous screening studies, there are still some uncertainties regarding the prevalence of premutation alleles in the general population. This is especially true for the reported prevalence of male carriers, which varies widely throughout the different published studies. Larger studies using highly sensitive methods are required in men of different ethnic origin to clarify this issue but it is likely that the premutation prevalence is much higher than reported in men.

Penetrance of FXTAS Among Premutation Carriers

A study of the penetrance of tremor and ataxia among adult carriers of premutation (*FMR1*) alleles, ascertained through families with known fragile X syndrome probands, showed that more than one-third of carrier men >50 years of age had both

tremor and ataxia. The penetrance increases with age, exceeding 50 % for men in their 70s and 80s (Jacquemont et al. 2004). This study, however, did not take into account allele size. Studies have shown that there is a correlation between motor involvement and age of onset of symptoms of FXTAS and that the penetrance of FXTAS may be lower at smaller allele sizes (Leehey et al. 2008; Tassone et al. 2007). This is of importance since small expansions represent the majority of premutation alleles (50 % of premutation alleles are 55–60 CGG repeats) (Jacquemont et al. 2006).

Prevalence of the Premutation in Movement Disorder Populations

All studies in movement disorder populations are summarized in Table 2.2. The ataxia populations tested had the highest rate of premutation alleles ascertained, but the studies had heterogenous inclusion criteria. Most of the studies ascertained patients who had negative gene testing for the spinocerebellar ataxias (SCA) and/or dentatorubropallidal luysian atrophy and Friedreich's ataxia. An additional two studies ascertained two late onset adult ataxia populations, adding *FMR1* screening as part of a panel of testing. Due to the difference in populations in these studies, screening in patients who have already tested negative for SCA or other gene tests yielded higher prevalence rates than patients who had not yet had testing done (1.7 % vs. 1.5 %). Hall et al. 2005) reported that only 4 % of people with FXTAS diagnosed in family studies had been evaluated by movement disorder neurologists, whereas the rest were seen by general neurologists or primary care physicians. Primary care physicians are less likely to order genetic testing for ataxia (e.g., only 1/70 medical charts reviewed in that study indicated spinocerebellar ataxia genetic testing). Thus, patients tested for *FMR1* in the movement disorder screening studies represent only a subgroup of patients with cerebellar ataxia, many of whom had been referred to tertiary referral centers for diagnosis.

Men referred for genetic testing for known mutations causing spinocerebellar ataxia (SCA) and for whom testing was negative were screened for repeat expansions in the *FMR1* gene (Brussino et al. 2005; Macpherson et al. 2003; Milunsky and Maher 2004; Van Esch et al. 2005). With a similar design, three additional studies included both men and women (Rajkiewicz et al. 2008; Rodriguez-Revenga et al. 2007; Zühlke et al. 2004). Three additional studies in Canada and the USA screened patients presenting with clinical features of adult onset cerebellar ataxia (Adams et al. 2008; Kerber et al. 2005; Kraft et al. 2005).

Table 2.2 Rate of *FMR1* repeat expansions in movement disorders

Movement disorder population	Total no. of patients	Estimated premutation rate (%)
Cerebellar ataxia	2014	1
Essential tremor	761	0
Multiple system atrophy	685	0.4
Parkinson disease	2901	0.3

The majority of the studies included subjects who (1157 men and 399 women) tested negative for SCA 1–3, 6–7, which account for approximately 65 % of autosomal dominant cerebellar ataxias worldwide (Brusse et al. 2007). Most of the other genes tested are much more rare. Thus, the populations of ataxia patients screened for *FMR1* are even more highly selected than what is typically seen in movement disorder clinics and may represent an overestimation of prevalence rates. The overall prevalence rate of *FMR1* premutations in the ataxic men ($n = 1532$) was 0–8.5 % and in the women ($n = 482$) was 0–3 %. These rates suggest that in men with cerebellar ataxia and negative SCA gene testing, the prevalence of *FMR1* repeat expansions may be as high as 8 %. However, the prevalence rate in women is unlikely to be higher than the rate in the general population. The median age of the men ranged from 48 to 65 years old although median age was not reported in eight of the studies. The median age of the women was not reported. Because the penetrance of FXTAS is age dependent, samples reporting median ages below or around 55 are likely to show an artificially low prevalence. The median age in the Flemish study of 65 may result in a more accurate reflection of the prevalence rate of manifesting premutation carriers (4 %) (Van Esch et al. 2005).

Despite case reports of patients with FXTAS presenting with an essential tremor (ET) phenotype, screening in this population has yielded virtually no cases. One screen was conducted in older adults presenting with ET and no premutation carriers were found among 40 men or 40 women (Garcia Arocena et al. 2004). A more recent study with 321 ET cases detected no premutation alleles in 154 men and 167 women (Clark et al. 2015). Several studies, reporting on groups of patients with different movement disorder diagnoses, included a total of 294 subjects with ET, diagnosed after the age of 45 and found no *FMR1* premutation range repeat expansion carriers (Deng et al. 2004; Tan et al. 2004). However, the phenotype screened in the studies excluded all subjects with parkinsonism and many required a familial history of tremor (autosomal dominant inheritance), making underestimation of FXTAS as a cause of tremor probable.

Some patients with premutation range expansions appear to have a Parkinson disease (PD) phenotype (Hall et al. 2009). To date, 2901 PD patients have been screened, with the majority being men (Annesi et al. 2004; Hall et al. 2011; Toft et al. 2005). Of these, five premutation carriers have been ascertained. Two smaller studies in patients with atypical PD and all types of parkinsonism did not yield any cases (Reis et al. 2008; Tan et al. 2005). Interestingly, Hedrich et al. (2005) looked at a larger population of parkinsonism patients with 265 men and 208 women and found one premutation carrier, who also had a second mutation in the *Parkin* gene. More recently, 595 Italian females (81 % with PD) were screened and two premutation carriers were identified (0.34 %) (Cilia et al. 2009). Although screening studies have not yielded many premutation carriers with parkinsonism, the majority of patients tested have met criteria for PD and unclassified parkinsonism patients constitute the minority. Because the phenotype of FXTAS would not typically meet criteria for PD, it is likely that parkinsonian FXTAS patients would be excluded from screening studies in idiopathic PD.

Prevalence rates are similar in multiple system atrophy (MSA) to those in PD, with a rate obtained in a large population of 507 men and women of 0.8 %, with most of premutation carriers having the cerebellar type of MSA (Kamm et al. 2005). Two other studies in American and Japanese MSA patients ($n = 141$) showed no premutation carriers (Garland et al. 2004; Yabe et al. 2004). In studies with mixed populations, only one woman with MSA and the premutation was found (Seixas et al. 2005; Tan et al. 2004).

Thus, screening for *FMR1* repeat expansions in movement disorder populations has resulted in different prevalence rates based on the population studied (Table 2.3) (Biancalana et al. 2005; Kraff et al. 2007). Premutation rates for ataxia of 0–4 % trend higher than rates reported in the general population of ~0.1 % (men). However, premutation prevalence rates in those subjects with essential tremor or parkinsonism are not increased compared to historical controls. This is despite data showing that patients with FXTAS are given diagnoses of tremor (20 %), ataxia (17 %), or parkinsonism (24 %) by their treating physicians (Hall et al. 2005). Many of the studies did not report mean age of the subjects, with some of the studies reporting mean ages less than 55 years. This likely underestimates the rate of premutation carriers, as most patients with FXTAS do not manifest symptoms until they are 60–70 years old (Jacquemont et al. 2004). In addition, the ethnicity of the subjects screened needs to be taken into account as prevalence rates of *FMR1* repeat expansions in the general population vary based on the ethnicity.

Most of the screening studies in movement disorders have been done in men due to original reports describing only affected men with FXTAS, making the premutation rates in affected populations of women less well defined. Overall, sample sizes were small relative to established prevalence rates in the general population. Most of the studies did not include a control group, but rather used historical controls. Further, techniques used to determine CGG repeat length in the *FMR1* gene vary from one study to another and are not reported in at least three studies.

Estimating the Prevalence of FXTAS

As mentioned earlier, there are no population-based studies on the prevalence of FXTAS. In 2008, the prevalence was estimated based on the following factors: (i) the prevalence of the premutation in the general population, (ii) the penetrance of FXTAS among premutation carriers, and (iii) the relationship between the premutation allele size and the penetrance of neurological signs in FXTAS (Hagerman 2008). For this estimate, a prevalence figure for the premutation of 1/800 for males of European origin was used and a figure of 40 % for cumulative penetrance of FXTAS in male carriers of the premutation. Also, the range of clinical involvement of FXTAS was restricted to those patients with premutation alleles >60 CGG repeats, which represents approximately 50 % (Table 2.2) of all premutation alleles (Jacquemont et al. 2006). Using these figures, the cumulative prevalence for men could be as high as 1 in about 4000, with this estimate subject to uncertainty of the

Table 2.3 Screening studies in movement disorders

Study	Author	Movement disorder	No. of patients	Population	Age—mean (SD)	Premutation rate in men	Premutation rate in women
Ataxia	MacPherson	SCA, 1, 2, 3, 6, 7, neg	59	British	uk	2/59	Nt
	Milunsky	SCA 1, 2, 3, 6, 7, 8, 10, 12, DRPLA, neg	167	American	uk (>50)	1/167	Nt
	Zuhlke	SCA 1, 2, 3, 6, 7, 12, 17, neg	510	German	uk (>50)	0/269	1/241
	Van Esch	SCA 1, 2, 3, 6, 7, neg	122	Flemish	64.9	5/122	Nt
	Brussino	SCA 1, 2, FRDA1, neg	275	Italian	48.3±14.2	6/275	Nt
	Kraft	Adult onset spinocerebellar ataxia	69	Canadian	uk	0/33	0/36
	Kerber	Late onset cerebellar ataxia	38	American	uk	0/20	0/18
	Rodriguez-Revenga	SCA 1, 2, 3, 6, 7, 8, DRPLA, neg	154	Spanish	uk	1/87	2/67
	Rajkiewicz	SCA 1, 2, 3, 6, 7, 8, 12, 17, DRPLA, neg	269	Polish	uk	1/178	0/91
	Adams	Mixed neg SCA, DRPLA	286	American	uk	1/286	Nt
Essential tremor	Arocena	Familial ET	81	American	76±20	0/40	0/41
	Clark	ET	321	American	uk	0/154	0/167
Multiple system atrophy	Garland	Gilman criteria[a]	64	American	65.9	0/40	0/24
	Kamm	Gilman criteria[a]	507	European	uk	2/253	2/254
	Yabe	Gilman criteria[a]	77	Japanese	uk	0/36	0/41
Parkinsonism	Toft	2/4 cardinal signs for PD	414	American	56.6	0/414	Nt
	Hedrich	Parkinsonism (UK Brain Bank)	473	German	uk	0/265	1/208
	Annesi	Idiopathic PD	203	Italian	67.7±8.6	0/203	Nt
	Tan	Idiopathic PD	121	Asian	uk	0/121	Nt

Mixed populations	Deng	Idiopathic PD or ET	412	American	PD 56.3, ET 53.7	0/412	Nt
	Tan	ET, SCA, MSA, atypical PD	367	Asian	Ataxia 50.3, MSA 56.5, ET 61, atypical PD 70	0/191	0/176
	Biancalana	Gilman criteria[a], OPCA, or CA; SCA 1, 2, 3, 6, 7, DRPLA, FRDA, neg	77	French	51.7	1/95 (CA)	1/28 (MSA)
	Seixas	SCA 1,2,3,6,7,8,12, HD, HDL2, DRPLA, neg	233	American	54.9±18	1/93	0/140
	Kraff	PD, DLB, FTD, MSA, PSP, CBD, ET	903	Italian	uk	3/903 (all PD)	Nt
	Reis	Tremor, ataxia, or parkinsonism	66	Brazilian	uk	0/66	Nt
	Cilia	PD, DLB, FTD, MSA, PSP, CBD, ET	595	Italian	55±11 (PD)	2/595 (all PD)	Nt
	Hall	ET, parkinsonism, ataxia	335	American	62.1±11 (men), 63.5±12 (women)	2/188 (ataxia)	0/147

SCA spinocerebellar ataxia, *neg* negative, *uk* unknown, *nt* not tested, *DRPLA* dentatorubropallidal luysian atrophy, *FRDA* Friedreich's ataxia, *ET* essential tremor, *PD* Parkinson disease, *MSA* multiple system atrophy, *OPCA* olivopontocerebellar atrophy, *CA* cerebellar ataxia, *HD* Huntington disease, *HDL* Huntington disease-like, *DLB* dementia with Lewy bodies, *FTD* frontotemporal dementia, *PSP* progressive supranuclear palsy, *CBD* corticobasalganglionic degeneration

[a]Gilman criteria are diagnostic criteria for MSA

overall prevalence of premutation alleles in the general population as well as the penetrance of FXTAS for a smaller premutation. Exclusion of slightly larger alleles, in the 60–70 repeat range (alleles >70 or 20 % of all premutation alleles), would predict a prevalence of about 1/10,000. Since this estimate was published, the prevalence of the premutation has increased, which would translate to a higher cumulative prevalence number. Also, these figures do not take into account the prospect of milder phenotypic involvement in carriers of smaller alleles. This uncertainty in the prevalence of clinical involvement among premutation carriers underscores the urgent need for additional screening studies on a larger scale and penetrance studies for smaller premutation alleles.

FXTAS in Women

There have been very few studies on women with FXTAS (Berry-Kravis et al. 2005; Hagerman et al. 2004). The symptoms appear to be milder in affected women and the penetrance appears to be much lower. In the families studied by Coffey et al. (2008), 15 of the 146 carrier women were found to have probable or definite FXTAS. However, 12 of these women were self-referred or were more likely to participate in the study due to the presence of neurological symptoms. If the self-referred women with FXTAS were eliminated in order to reduce ascertainment bias, a total of 6 women out of 134 (4.5 %), or 6 out of 72 women over age 40 (8.3 %), had FXTAS. With the same approach described previously, the prevalence of FXTAS in Caucasian women would be estimated using a premutation prevalence of 1/300, a hypothetical penetrance of FXTAS of 1/13, and a clinical involvement in alleles >60 CGG repeats (50 % of all alleles), and the resulting prevalence rate would be 1/7800 ($1/300 \times 1/13 \times 1/2 = 1/7800$).

Summary

Although there is growing epidemiological data on the premutation, the prevalence of FXTAS continues to warrant epidemiological study. Based on estimates derived from the prevalence of the premutation allele and the penetrance of FXTAS, it seems that this new disorder may be one of the more commonly known single gene neurodegenerative diseases. However, studies of movement disorders populations, of which there are now 27, report that the gene abnormality is not associated with a significant number of movement disorder cases. This may be consistent with the fact that a large proportion of FXTAS patients are not followed in movement disorder clinics, which has been confirmed in prior studies (Hall et al. 2006). Premutation carriers may be excluded from cohorts that are screened for the gene mutation, such as those mentioned above. FXTAS shows age-dependent penetrance and the mean age in many of the screening studies in movement disorders is close to 55, which likely

reduced ascertainment. Individuals with milder movement disorders secondary to the premutation may not have met inclusion criteria for spinocerebellar ataxia, parkinsonism, or ET when seen in clinic. For example, criteria for ET requiring a first-degree relative with essential tremor may have caused underestimation of *FMR1* repeat expansion rates in a tremor population. Diagnostic criteria for idiopathic PD would exclude many patients with FXTAS, since they would have cerebellar ataxia and kinetic tremor.

In summary, the present literature suggests that the prevalence of FXTAS in males of the general population is in the range of 1/3500–1/4500. This is based on the current estimates of prevalence of the premutation allele in the general population, which may be more prevalent than currently available data indicate. *FMR1* premutation alleles are increased in ataxia populations, but due to the heterogenous clinical presentation of FXTAS, genetic screens have failed to identify a large proportion of premutation carriers in any given movement disorder population. Ongoing FXTAS neurological phenotype–genotype studies may better clarify the spectrum of patients who should be tested for *FMR1* repeat expansions. Ethnicity needs to be taken into account when screening populations in the future, due to disparate *FMR1* premutation prevalence rates. Although guidelines for testing have been proposed (Hall et al. 2005), a larger cross-sectional study with a broader range of movement disorder phenotypes would be ideal to provide the best foundation for guidelines in the future.

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Chapter 3
FXTAS: Neuropsychological and Neuropsychiatric Phenotypes

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Abstract This chapter reviews research on the neuropsychological and psychiatric signs and symptoms of the fragile X-associated tremor/ataxia syndrome (FXTAS). We summarize what is known about cognition and psychological/psychiatric functioning in affected individuals, as well as asymptomatic carriers of the *FMR1* premutation. The neuropsychological impairment associated with FXTAS primarily involves impaired executive functioning. Hence, one frequently observes disorders of behavioral self-regulation, planning, inhibition, working memory, and information processing. These deficits appear to interact with, and contribute to, comorbid psychiatric symptomatology such as anxiety, depression, impulsivity, apathy, irritability, and agitation. Most studies to date have been cross-sectional, and it is difficult to draw inferences regarding the progression and timing of cognitive decline in FXTAS. Moreover, as some individuals with early stage FXTAS have minimal or no cognitive/psychiatric impairment, even in the presence of significant neurological deficits, these manifestations of the disorder may be quite heterogeneous. Further research will enhance our understanding of the temporal and symptomatic relationships between neuropsychological and neurological findings, and of other genetic, epigenetic, and environmental variables that determine the development, course, penetrance, and severity of FXTAS.

Keywords Fragile X-associated tremor/ataxia syndrome • Cognition disorders • Movement disorders • Fragile X premutation • *FMR1* gene

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Introduction

FXTAS was identified serendipitously in 1999 in the context of a genotype–phenotype study of families of children with Fragile X syndrome (FXS) (Hagerman et al. 2001). The first studies of FXTAS had a number of important limitations. In the beginning, the incidence and prevalence of this recently identified disorder were entirely unknown, so it was uncertain whether FXTAS was a distinct entity associated with *FMR1*, and whether it affected very many people. Moreover, the FXTAS phenotype was not well understood, and the natural history of the disorder was unknown.

Few people had heard of FXTAS, and many confused it with Fragile X syndrome (FXS), which had been identified in 1943 by Martin and Bell, and which was at first called the Martin–Bell syndrome. The fragile site on the long arm of the X chromosome was discovered by Lubs in 1969, and the FMR1 gene itself was later located by Verkerk et al. (1991). While an association between a defect on the X and intellectual disability had been known since the paper by Martin and Bell, no association with a movement disorder had been noticed in families with a child who had FXS. Furthermore, carriers of the fragile X premutation had not been observed to be at risk for tremor and ataxia, or for any but subtle cognitive anomalies (see the review of the issue of cognition in (Moore et al. 2004a). Some investigators therefore doubted the existence, or at least the public health significance, of the disorder.

Further complicating things were differences in how the premutation, and FXTAS, affect men and women. The phenotype of FXS, which is associated with a full mutation of *FMR1*, is more severe for males than females. Therefore, it was expected that the situation would be similar for carriers of the premutation, as the presence of a second X chromosome among heterozygous females would presumably protect them from developing FXTAS. Because of the small number of male cases and the absence of females with FXTAS, early research focused almost exclusively on men. This research was influenced by an obvious ascertainment bias, as FXTAS was typically identified in the clinic among the grandfathers of girls with FXS, and not in community-based studies. Such studies were of course impossible because prior to the identification of FXTAS, neurologists—even those specializing in movement disorders—had never heard of it, and all people with the disorder were therefore misdiagnosed, and unavailable for participation in research. Hence, for quite some time after FXTAS was identified, it was very difficult to recruit subjects outside a Fragile X clinical or research setting. This meant that the few available older men who were known to have the tremor/ataxia syndrome were in demand as research subjects. They were frequently studied and were typically examined multiple times in research projects conducted at different sites.

The field, and the research, have matured considerably. Although FXTAS is still not widely known, and a significant percentage of affected individuals continue to be incorrectly diagnosed, the signs, symptoms, course, and biology of the neurologic disorder are better understood. In some respects, FXTAS now appears to be the neural expression of a complex premutation phenotype that affects psychiatric,

immune, and endocrine functioning. It also may have both neurodevelopmental and neurodegenerative aspects, as has been suggested by several investigators (e.g., (Battistella et al. 2013).

Although the cardinal features of FXTAS have been characterized, their expression is variable, and many of the factors influencing onset, severity, and progression remain unknown. The most obvious clinical features of FXTAS are action tremor and gait instability, from which the disorder derives its name, but cognitive and psychiatric/psychological disturbances are very prevalent among affected persons. With few exceptions (e.g., Leehey et al. 2007, described in Chap. 1), the natural history of FXTAS has not been studied, and there is much to learn regarding the clinical and temporal relationships between the motor, cognitive, and psychiatric signs and symptoms. Cognition may be intact in the earliest stages of FXTAS although it is likely that some subtle impairment often precedes the appearance of the movement disorder (Hunter et al. 2012). As FXTAS advances, cognitive deficits become very common, and worsen over time. Dysexecutive functioning eventually emerges as a cause of severe functional disability (Brega et al. 2009); however, such impairment is considered one of the minor diagnostic criteria of FXTAS (Jacquemont et al. 2003). In this review, we summarize current knowledge about cognitive and psychiatric functioning in people with the premutation—both those who have FXTAS, as well as people who carry the premutation but have no clinical signs of neurologic disorder.

Executive Function and Dysexecutive Syndrome

Executive functioning (EF), an aspect of cognition that is significantly impaired in FXTAS, is the capacity for the autonomous regulation of one's own attention and behavior, especially in the service of one's intentions (Fuster 2000; Luria 1980; Quintana and Fuster 1999). This functional system has a number of subcomponents including planning, maintaining and appropriately shifting attention, initiation of purposeful behavior, inhibition of inappropriate behavior, the ability to engage in effortful activity, and critically and accurately monitoring the performance and outcomes of one's activity within specific contexts (insight), among others. Impairment of any part of the system can result in a *dysexecutive syndrome* that may have different manifestations depending on the specific functional subcomponents that are affected.

EF is an emergent property of a widely distributed functional system involving the cerebral cortex, especially parts of the prefrontal cortex (PFC), and several subcortical structures. The executive networks consist of several important nodes and the connections among them. In addition to dorsolateral PFC, these include the anterior cingulate cortex, supplementary motor area, cerebellum (especially the dentate nuclei), basal ganglia, and parts of the thalamus (Fiez 1996; Goel and Grafman 1995; Grafman 1995; Hallet and Grafman 1997; Jenkins et al. 1994; Leiner et al. 1993; Middleton and Strick 2001; Nichelli et al. 1996; Raichle et al.

1994; Roland 1993). The "supraordinate" contribution of PFC to executive functioning, according to Fuster (Fuster 1997, 2000; Quintana and Fuster 1999) is "the formation of temporal structures of behavior with a unifying purpose or goal—in other words, the structuring of goal-directed behavior." The idea is that the PFC integrates declarative memory with prospective memory (i.e., remembering to remember in the future) in working memory (the contents of awareness in the present moment).

Dysexecutive syndromes (Baddeley 1986) are characterized by specific kinds of behavioral pathology. These include distractibility, impulsive behavior that is irrelevant or inappropriate in a given situation, perseveration, apathy, and failure to undertake purposeful activity, all of which reflect varying degrees and types of dissociation between volition and action (Luria 1980). An individual with a dysexecutive syndrome demonstrates a disruption of the coherence and integrity of deliberate behavior. This is a central issue in the FXTAS phenotype.

Many abilities that are not, strictly speaking, executive in nature rely on EF for their appropriate activation and maintenance (e.g., procedural learning; Beldarrain et al. 1999). This is especially the case in circumstances that are novel, complex, or threatening. The performance of almost any cognitive task, as it becomes increasingly complex, is dependent on the integrity of EF. Hence, persons with FXTAS are likely to have difficulty with tests of declarative learning. This does not appear to be the type of problem seen in persons with amnestic disorders affecting hippocampal memory, but rather reflects shortcomings in active learning and retrieval, which are associated with dysexecutive cognition (Brega et al. 2008).

Epidemiology of FXTAS and the *FMR1* Premutation: Gender Differences

When FXTAS was first identified among a small group of older men (Hagerman et al. 2001), it was unclear whether females were affected by the disorder. Initially, only male carriers of the premutation were found to have the neurological disorder, but it is now known that female premutation carriers also may develop FXTAS. Nevertheless, there are clear sex differences in the incidence, prevalence, and expression of the disorder.

The likelihood that a female carrier of the FMR1 premutation will be affected by FXTAS (i.e., penetrance) is lower than that for male carriers, and although there exist estimates of penetrance for both sexes (Coffey et al. 2008; Hagerman and Hagerman 2004a; Jacquemont et al. 2004b), the true rate remains unknown. In addition, the prevalence of FXTAS in the general population can only be extrapolated from currently available prevalence figures for FMR1 premutation alleles in the population overall, along with penetrance data. The best prevalence estimates of the premutation in the USA are 1 in 148 to 1 in 209 females, and 1 in 290 to 1 in 430 males (Maenner et al. 2013; Tassone et al. 2012b) (see Chap. 2 for more detail).

The female clinical phenotype also appears to differ in certain respects from that observed in males (Berry-Kravis et al. 2005; Coffey et al. 2008; Hagerman et al. 2004b; Jacquemont et al. 2006). One likely determinant of these gender differences is X-inactivation among women (Berry-Kravis et al. 2005; Jacquemont et al. 2005), in which one or the other X chromosome in each of a woman's cells is inactivated. This process limits the total dose of proteins that would be produced by genes on the X if both X chromosomes were active. Hence, X-inactivation reduces the fraction of cells that contain an active expanded (abnormal) FMR1 allele. The process of X-inactivation is thought to be largely stochastic; a favorable activation ratio (AR), which is present when >50 % of the chromosomes bearing defective genes are inactivated, is more likely to yield a normal phenotype than an unfavorable AR (Berry-Kravis et al. 2005; Jacquemont et al. 2005).

Women also appear more likely than men to have comorbid pathology affecting other physiological systems. For example, female carriers of the premutation have an increased likelihood of developing Fragile X-associated Primary Ovarian Insufficiency (FXPOI), which causes them to experience menopause in their late 30s or early 40s. The high incidence of primary ovarian insufficiency (POI) among female carriers (Allingham-Hawkins et al. 1999; Murray et al. 2000; Sullivan et al. 2005) suggests anomalous development of the ovaries and, perhaps, of other aspects of the endocrine system. This could be significant given that it is thought that estradiol may be neuroprotective in such conditions as Alzheimer's disease (AD; e.g., Asthana et al. 1999; Behl and Holsboer 1999; Woolley 1999). Moreover, the estrogenic steroids appear to affect specific aspects of cognitive functioning (especially verbal fluency and verbal memory; e.g., Grodstein et al. 2000; Kimura 2000; Kimura and Hampson 1994; Sherwin 1988; Sherwin 1994a, b; Sherwin 1999). This raises the possibility, not yet investigated, that female carriers of the premutation who experience FXPOI may be at increased risk of Alzheimer's disease and other cognitive problems as they age. There is no evidence, however, that FXPOI represents a risk for the motor symptoms of FXTAS (Coffey et al. 2008).

There is reason to hypothesize that neuroendocrine variables may influence the male phenotype as well, given the report by Greco et al. (2007) of inclusion bodies in Leydig cells of the testes of two men with FXTAS, and in both the anterior and posterior pituitary of one of these cases (a finding also noted by Louis et al. (2006). As testosterone is thought to have effects on circumscribed aspects of cognition, especially working memory and visual–spatial performance (Janowsky et al. 2000), the impact of neuroendocrine changes associated with the premutation may contribute to the cognitive decline directly associated with neurodegeneration.

Female carriers also appear to be susceptible to a number of other disorders, several of which affect the immune system in particular (e.g., fibromyalgia and autoimmune hypothyroidism; Coffey et al. 2008). Interesting in this regard is the fact that both fibromyalgia and clinical hypothyroidism appear to have effects on both cognition and mood that are at least superficially similar to those that accompany FXTAS (Hennessey and Jackson 1996; Mease 2005; Tesio et al. 2015), and that inflammation may play a role in all of these disorders (Ashwood et al. 2010; Greco et al. 2008; Marek et al. 2012; Winarni et al. 2012).

Cognition Among Male Carriers of the Premutation: Case Studies and Case Series

Numerous papers have addressed cognition among men with FXTAS. Initial reports focused on a small number of men and often were limited in the scope of cognitive functioning addressed. However, these early studies strongly suggested that cognition was significantly affected by FXTAS.

In the initial report of a series of five male cases (Hagerman et al. 2001), three individuals showed marked impairment of nonverbal intelligence, obtaining performance IQ scores between 1.5 and nearly 3 standard deviations below the mean on the Wechsler Adult Intelligence Scale—Third Edition (WAIS-III; Wechsler 1997a). Verbal functioning was less affected, but perhaps the most striking finding was that all five cases demonstrated significantly impaired EF.

Subsequent case reports and case series have been limited in scope and in the thoroughness of cognitive assessment, and the results were consequently somewhat mixed. Rogers et al. (2003) used semi-structured telephone interviews of spouses, obligate carrier daughters, and affected individuals to assess the neurological and cognitive status of a group of grandfathers with the premutation, most of whom had tremor (80 %) and ataxia (55 %). They reported that "failing intellect was described in one-third of cases, with early loss of memory for recent events, increasing social incompetence and ultimately dementia" (p. 55). Another paper reported on the examination of two brothers who were premutation carriers (Peters et al. 2006). One demonstrated impaired performance on the Mini Mental State Exam (MMSE; Folstein et al. 1975) and the Stroop test (a measure of EF), and a mildly impaired performance on the Mattis Dementia Rating Scale (Mattis 1988), in association with significant tremor and ataxia. His sibling, who may not have been examined formally, had only mild neurological signs, and no mention was made of any cognitive impairment.

Mothersead et al. (2005) discussed the case of a man who died at 67 years of age following a rapidly deteriorating clinical course. He had tremor and gait ataxia and showed significant neuropsychological impairment across a wide range of tasks. Pathologic examination of his brain revealed the characteristic inclusion bodies of FXTAS, as well as histological evidence of intermediate-stage Alzheimer's disease. The authors suggested that the rapid progression of his clinical condition might be a function of his being affected by two coexistent disease processes.

The male FXTAS phenotype shows some heterogeneity, as illustrated by several other cases. For example, Gonçalves et al. (2007) reported a case of rapidly progressive dementia in a 70-year-old man who was thought to have developed severe dementia over a period of about 1 year. In comparison with his cognitive deficits (MMSE = 11/30, indicative of severe dementia), his movement disorder was relatively mild. His MRI, however, showed diffuse cerebral and cerebellar atrophy and the hyperintensities of the middle cerebellar peduncles (MCP) that are a frequent characteristic of FXTAS. No baseline examination was available, and the authors dated the onset of symptoms to a report that the patient had begun to demonstrate reclusiveness and apathy about a year prior to their evaluation.

The cases reported by Capelli et al. (2007) also demonstrate the range of symptomatology among men affected by FXTAS. Four brothers, three of whom were carriers, were examined. All three carriers had a positive MCP sign although one was essentially asymptomatic. Of the two with clinical signs of FXTAS, one (with 4 years of education) obtained an MMSE score in the normal range (27/30), while the other, who had gone to school for 8 years, scored 18 on the MMSE, a score suggesting significant cognitive impairment.

One case from a series of four individuals reported by Loesch et al. (2008) also deserves mention. This 52-year-old man had mild ataxia and marked white matter hyperintensities in the MCPs bilaterally. Although he showed normal intelligence—obtaining a full-scale IQ score above the population mean on the WAIS-III—this patient had a Working Memory Index score about one standard deviation below the mean on the WAIS-III. Noteworthy in this case was the severity of the MCP lesions in an individual with minimal neurological signs of FXTAS.

Only one case report presents the results of a relatively thorough neuropsychological examination of a man with FXTAS (Grigsby et al. 2006a). The patient, who had 98 CGG repeats, was one of the five original cases of FXTAS discussed by Hagerman et al. (2001). He was followed from his identification at age 64 until his death 5 years later. Onset of FXTAS for this patient, who had 98 CGG repeats, was at age 54, with action tremor in his dominant right hand. By the age of 61, he required assistance with dressing and eating. The initial neurological exam (Hagerman et al. 2001) showed significant cerebellar and Parkinsonian motor deficits (masked facies, impaired saccades, slow intention tremor without dysmetria, postural tremor, and intermittent resting tremor). His gait was slow and wide based. By the age of 67 years, he was progressing rapidly; he was unable to walk independently, and had chronic urinary and bowel incontinence. He spent 2 years in a wheelchair before his death at age 69.

During the neuropsychological examination at age 64, he was disinhibited and distractible, with inappropriate joking and perseverative language and thinking. Attention and information processing were impaired, with distractibility and difficulty shifting attention. Age-adjusted information processing speed ranged from the first to the ninth percentile. Working memory was moderately to severely impaired, and at times he forgot the instructions before beginning a test. On tests of EF his performance ranged from two to five standard deviations below the mean. Both procedural and declarative learning were significantly impaired; his performance on tests of word list learning and paragraph recall was at about the 2nd percentile immediately and after a 20′ delay. He showed mild ideomotor apraxia; focal cortical cognitive deficits are unusual in FXTAS, but there are unpublished reports of apraxia in a number of advanced-stage FXTAS patients.

The extreme impairment of EF and working memory stood in contrast to his performance on verbal tests. His verbal IQ (VIQ) of 93 was 0.5 standard deviation (SD) below the mean, but nevertheless in the average range. His WAIS-III nonverbal (performance) IQ (PIQ) was 73, nearly 2 SD below the mean, presumably as a consequence of tremor, deficits in working memory, impaired EF, and reduced processing speed. Examined again 4 years later, approximately 12 months prior to

death, little formal assessment was possible, and only three measures of EF could be completed. On these, his performance had declined by up to two standard deviations.

Neuropsychological Phenotype of Men with FXTAS

The initial studies of cognitive impairment in FXTAS focused on executive functioning, working memory, learning, and general intellectual ability. Sample sizes were typically small, and comparison groups were either lacking or limited in different respects. The first, preliminary study examined 25 men with FXTAS (Grigsby et al. 2006a). The average age of onset of both tremor and ataxia was at 61 years. The mean age at the time of the study was about 71 years, so the average duration of FXTAS was approximately 10–11 years. The mean education was 16.3 years (range = 12–21), and the mean number of CGG repeats was 85 (range = 55–102). There was no comparison group; instead, subjects were compared with normative data on several tests including the WAIS-III, three measures of EF, and the Symbol Digit Modalities Test (SDMT; Smith 1968), a test of information processing speed.

Scores on two measures of EF showed substantial impairment. The capacity for inhibition (assessed by the Stroop test) was severely affected in one-quarter of the sample, and processing speed was profoundly impaired in most subjects. Although verbal and performance IQ scores were not significantly different from the general population mean (means = 102.4 and 93.4, respectively), they were quite low given the sample's educational level. Correlations between individual measures of cognition and CGG repeat size ranged from −0.23 to −0.55. The results supported the hypothesis that FXTAS involves marked impairment of EF, with a less striking effect on global measures of intelligence. In addition to the small number of measures, the lack of a control group was a significant limitation of this study.

A comparison of 33 males with FXTAS and 25 men with a normal *FMR1* allele was published the following year by Grigsby et al. (2007). Subjects with FXTAS, recruited through a genotype–phenotype study of FXS, met the criteria for definite or probable FXTAS (Jacquemont et al. 2003). Fourteen FXTAS subjects had been subjects in the study discussed above (Grigsby et al. 2006a). The mean age was 69.8 for the FXTAS group and 64.6 for controls, so analyses were adjusted for age. The mean number of CGG repeats for FXTAS subjects was 92 (range = 62–130). Comparison subjects were recruited from the community. The mean age of onset of ataxia was 61.4, and onset of tremor was 62.1. Three comparison subjects (11 %) had a mild kinetic or postural tremor, which is roughly equivalent to the prevalence in the general population for older males; none were ataxic. Measures used included the WAIS-III (IQ scores and summary indices), four tests of EF, and the SDMT.

Adjusting for age and education, both verbal and nonverbal IQ scores were impaired for FXTAS subjects although the mean VIQ of individuals with FXTAS (101.7) was near the mean for the general population; the comparison group scored about one standard deviation above the mean (116.3). The difference was more

marked for PIQ (FXTAS mean = 89.3, control mean = 112.0). Nonverbal subtests of the WAIS-III rely more heavily than the verbal subtests on processing speed and manipulation of objects with the hands, likely contributing to the greater difference between groups. FXTAS subjects also had lower scores on WAIS-III verbal comprehension, perceptual organization, and working memory indices. Similar findings were observed on three of the four EF measures, although differences on the Stroop fell short of significance, but a subset of FXTAS subjects (22.7 %) performed two or more SD below the control group mean. No comparison subjects obtained scores in that range. Finally, processing speed was significantly worse for the FXTAS group on both the SDMT and the WAIS-II processing speed index. As in the previous study, correlations between CGG repeat size and individual test performance ranged from −0.30 to −0.55. Level of mRNA was significantly and inversely associated with several cognitive measures, including PIQ, two measures of processing speed, and two tests of EF.

The first comprehensive study of neuropsychological functioning among males with FXTAS (Brega et al. 2008; Grigsby et al. 2008) compared groups of individuals with probable or definite FXTAS ($n = 42$), asymptomatic carriers ($n = 28$), and men with a normal FMR1 allele ($n = 39$). Subjects with FXTAS were significantly older (mean = 68.1 years) than asymptomatic carriers (mean = 59.1 years), but not different than normal controls (mean = 63.5). This age difference between symptomatic and asymptomatic carriers may reflect age-dependent penetrance in FXTAS (Jacquemont et al. 2004b; Tassone et al. 2007), as younger individuals who carry the premutation may not yet have become symptomatic, but may convert to FXTAS as they age. This age-related systematic biasing of the premutation sampling frame is an interesting methodologic issue in studying FXTAS, as at any time an unknown percentage of the asymptomatic carriers is developing preclinical disease. It may even be that this subset of persons accounts for some portion of the findings of subtle impairment reported among carriers without FXTAS.

In any case, baseline imaging for several members of the asymptomatic carrier group showed cerebellar volume loss and hyperintense signal in the MCPs, suggesting that those individuals may have been in early preclinical stages of FXTAS. This would be consistent with the findings of Loesch et al. (2007), who noted preclinical radiologic evidence of FXTAS among individuals with negligible neurological signs.

The examination involved administration of a wide range of cognitive and neuropsychological measures, a neurological evaluation conducted by a neurologist specializing in movement disorders, magnetic resonance imaging, and molecular (CGG and mRNA) analyses. As in previous studies, the neuropsychological examination involved the WAIS-III, as well as tests of EF and processing speed. In addition, subjects were administered the MMSE, tests of word learning and story recall, speech and language, and fine motor functioning, which was used as a covariate to control for the effects of tremor on performance.

FXTAS subjects performed significantly worse than persons with a normal allele on the MMSE, VIQ, and PIQ. For the MMSE, the absolute difference was small (1.6 points), and both groups were in the normal range, a result consistent with the

findings of Bacalman et al. (2006), who had a relatively small sample, but found few persons with FXTAS who had MMSE scores in the impaired range. On the WAIS-III, both groups were in the average to superior range, but differed by approximately a full standard deviation. The normal allele group had a mean VIQ of 120.7 compared with 106.6 for the FXTAS group, while the mean PIQ scores were 116.4 and 97.7, respectively. In contrast with the results for the MMSE, these differences were clinically meaningful, but it is important to note that after a mean duration of illness of roughly a decade, IQ scores of men with FXTAS remained at about the population mean, although given that the groups had similar levels of education, it seems probable that they had declined by as much as a standard deviation. This is a very different pattern than that observed in AD, in which a much greater decline in IQ, into the low average range, would be anticipated.

There were no differences between subjects with FXTAS and controls on any language measures, a finding also at variance with what would be expected in AD. Consistent differences were found, however, on measures of EF ($p < 0.0001$), working memory ($p < 0.001$), information processing speed ($p < 0.01$–0.0001 on different measures), and immediate recall ($p < 0.01$–0.001). Interestingly, the percent of material retained after a 30′ delay did not differ significantly although individuals with FXTAS had consistently lower scores than did controls (e.g., 79 % vs. 92 % on the Logical Memory Test of the Wechsler Memory Scale-III). Finally, the groups did not differ on verbal reasoning, as measured by three subtests of the WAIS-III (Vocabulary, Similarities, and Comprehension). Again, subjects with FXTAS had nonsignificantly lower scores, also inconsistent with the pattern observed in AD.

Brega et al. (2008) attempted to clarify the nature of the dysexecutive syndrome that characterizes FXTAS, and to assess the role of EF as a mediating variable on tests that rely on, but do not directly measure, EF. For example, the pattern of results on memory testing suggested that the primary difficulty was not with memory consolidation per se, but resulted from EF deficits. Delayed recall, after all, was not as impaired as would be seen in AD. The investigators hypothesized that this might be due to difficulty making a deliberate effort, secondary to impaired EF.

Exploring in more depth the data reported by Grigsby et al. (2008), they added several more recently enrolled subjects, yielding a sample of 47 FXTAS subjects, 32 asymptomatic carriers, and 41 controls. Two sets of analyses were performed on the data, the first examining in greater detail group differences in EF between the two carrier groups and controls. The other set of analyses assessed the extent to which EF was responsible for group differences across the entire set of measures for which significant differences were found by Grigsby et al. (2008). A new set of regression analyses was conducted to ensure that the previously reported findings were stable; the results remained essentially the same. The mediational analyses then conducted used ordinary least squares regression with nonparametric bootstrapping for estimation of confidence intervals (CIs). For all dependent variables, they computed CIs for sampling distributions including 2000 and 5000 samples drawn randomly from the available data.

The FXTAS sample performed significantly worse than men with a normal FMR1 allele on 17 of the 19 individual measures of EF used in the study (including

the nine individual items of one measure, the Behavioral Dyscontrol Scale; Grigsby et al. 1992). These results suggest that FXTAS apparently causes impairment of most aspects of EF, including working memory (remembering intentions), inhibiting incorrect or inappropriate responses, control of attention, initiation of appropriate responses, and monitoring the accuracy of their performance (i.e., insight).

The nonparametric bootstrapping mediation analyses yielded support for the hypothesis that EF deficits lead to poor performance on many cognitive measures that are not explicit tests of EF. Irrespective of the EF measure used as a mediating variable, EF accounted for differences in performance between FXTAS and normal allele groups on the MMSE, VIQ, PIQ, WAIS-III Information subtest, performance on a word learning test, and processing speed. For other measures of cognitive functioning (immediate and delayed declarative memory, and the WAIS-III Block Design and Picture Arrangement subtests), two or three of the four measures of EF mediated the effect of group on performance.

Neuropsychological Phenotype of Asymptomatic Male Carriers

Although there are some contradictory findings, there seems to be a consensus that at least a subset of men who have the FMR1 premutation, but who do not have FXTAS, have difficulties with executive functioning (Hunter et al. 2008). There is some inconsistency in the literature, but there is no evidence of cortical or more global impairment of cognition (Hunter et al. 2009).

Loesch et al. (2003) were among the first to report impaired EF among asymptomatic carriers of the premutation. Work by Cornish et al. (2005) found evidence of a relative weakness in social cognition among carriers. In later studies of unaffected carriers, Cornish and her colleagues (Coffey et al. 2008; Cornish et al. 2009, 2011) demonstrated age-related declines in the executive capacity for inhibition and working memory in excess of what is observed among age- and IQ-matched men with a normal *FMR1* allele. These declines appear to begin in early adulthood and continue across the lifespan, and they may be more significant for individuals with >100 CGG repeats (Cornish et al. 2011).

Moore et al. (2004a) studied a group of 20 mostly middle-aged male carriers in comparison with a group of 20 males with normal alleles. The groups, which were similar on both age and IQ, differed from one another on several measures of EF, remote memory, and declarative learning. These results were consistent with their own study of cortical and white matter volumes among the same subjects (Moore et al. 2004b). In that study, Moore and her colleagues found lower volumes in a number of areas of the brain, including amygdala, hippocampus, left thalamus, cerebellum, genu of the corpus callosum, and others.

EF deficits were reported in a larger study by Grigsby et al. (2008). After adjusting for age and education, asymptomatic premutation carriers obtained nonsignificantly lower mean scores than controls on most tests, but significant differences

were found on an EF composite score, and both immediate and delayed recall on the Logical Memory Subtest of the Wechsler Memory Scale-III (Wechsler 1997b). In general, it appears that asymptomatic male carriers as a group have a slight EF deficit compared with persons who have a normal *FMR1* allele. In light of findings of preclinical radiological indicators of FXTAS, it may be that a subset of these individuals is developing FXTAS and that executive functioning is mildly impaired prior to the development of frank neurological signs. This might go undetected much of the time, as early EF deficits are frequently interpreted as personality change or a reaction to difficult environmental factors. It is also possible that carrier status itself is associated with some subtle difficulty in EF, independent of the risk of developing FXTAS.

One shortcoming of this line of research with asymptomatic carriers is the variability in sampling strategies and measures of cognition that have been used by different investigators. To deal with this issue, Hunter and her colleagues at different research centers pooled and analyzed the data from their respective samples. Taking advantage of the resulting sample of 100 asymptomatic male carriers of the premutation and 216 comparison subjects who had normal alleles, they conducted analyses using the measures of working memory and inhibition they had in common. Subtle differences, of minimal clinical significance, were detected on the Stroop test, but there were no significant between-groups differences on the other measures.

In their recent review of the literature, (Grigsby et al. 2014) concluded that the evidence provided support for the existence of "a subtle, suboptimal level of cognitive performance among a subset of unaffected carriers" that involved deficits in executive functioning and working memory, in a subset of men who have the premutation, but who have no clinical signs of FXTAS.

Neuropsychological Phenotype of Women with FXTAS

Our knowledge of cognition among females who are carriers of the *FMR1* premutation has some significant gaps. Female carriers who have no signs of FXTAS have been studied systematically, but this is not the case for women with FXTAS, among whom there have as yet been no systematic studies of neuropsychological functioning. Therefore, while we have a good understanding of the FXTAS phenotype among men, the female phenotype is less well known. What we do know about how the disorder affects women's cognitive abilities comes largely from case reports and case series.

Hagerman et al. (2004b) reported a case series of five women with FXTAS, all of whom obtained IQ scores in the average to superior range, although one had a nonverbal IQ about one standard deviation below the mean (low average). Two exhibited mild deficits in EF, and one reported mild memory problems that she considered consistent with her age, which was in the early 60s. As these were cases seen in clinic rather than in a research protocol, the neuropsychological data obtained were

neither consistent nor comprehensive, but suggested some similarities with what is observed among men.

Zuhlke et al. (2004) discussed the case of a 73-year-old female with onset of the motor signs of FXTAS at 64, who experienced ataxia, action tremor, and significant lower extremity weakness. The patient complained of problems with "memory and concentration," and obtained a score of 25/30 on the Mini-Mental State Examination (MMSE). The lack of educational history or a previous MMSE, and the fact that the MMSE is a relatively blunt instrument for assessing mild cognitive declines, makes this finding difficult to interpret, but suggests impairment of some aspect(s) of cognition. The MMSE score was lower than the means reported in Grigsby et al. (2008) for men with a normal *FMR1* allele (29.6), asymptomatic carriers (29.2), and men with FXTAS (27.2).

Berry-Kravis et al. (2005) reported on two sisters aged 77 and 83, with CGG repeat sizes of 69 and 83, respectively, who had both experienced the onset of symptoms 2 years prior to their neurologic examinations. The activation ratio (AR) favored the normal allele in the older sister, and the expanded premutation allele in the younger, who had a more significant movement disorder than her elder sibling. Neither patient underwent extensive cognitive assessment, but the authors stated that neither had "dementia." While that may be the case, the brief duration of illness for both when examined—only 2 years—would make the presence of significant cognitive impairment unlikely, assuming the rate of progression of the disorder among women is similar to that among men.

A clinical report by Horvath et al. (2007) concerned a 68-year-old woman who had developed the first signs of FXTAS about 8 years previously. She had 95–105 CGG repeats, and experienced menopause at age 39, which together suggest she had the FXPOI that affects a significant percentage of female *FMR1* carriers. The patient "complained of deterioration of her memory and concentration," and an unspecified neuropsychological exam was summarized as having found "a slight memory deficit, decreased mental flexibility, and significant anxiety." The length of time since onset of tremor and ataxia was consistent with the development of cognitive problems associated with FXTAS—she had mild difficulty with memory, and the "decreased mental flexibility" may have been a manifestation of impaired EF.

Jacquemont et al. (2005) reported on a pair of sisters with what may have been an atypical variant of FXTAS, involving spastic paraparesis, intention tremor, peripheral neuropathy, limb ataxia, and abnormal muscle biopsies. Onset for both was at about the age of 30, which is unusually early for FXTAS. A number of other genetic etiologies were ruled out, but assuming other factors do not account for the spastic paraparesis and muscular wasting, the course and signs of their neurologic disorder may reflect unusual diversity in the presentation of FXTAS. Both were said to be cognitively intact, but no details were provided.

The interpretation of two published case reports describing cognitive deficits has been complicated by the presence of comorbid neurologic or other medical conditions and their treatment. For example, O'Dwyer et al. (2005) wrote of a woman with FXTAS who also had breast cancer with liver metastases. By day 11 of chemotherapy with carboplatin and docetaxel, there was a marked exacerbation of her

existing mild movement disorder. She rapidly became severely ataxic, unable to walk or stand without assistance. After discontinuation of carboplatin, she and her husband thought the tremor and ataxia had gradually improved to her pretreatment status. Resumption of docetaxel on day 42 had no apparent effect on her functioning, although nearly a month later a cognitive screening exam showed mild global cognitive impairment, and a significant dysexecutive syndrome. The severe but transient increase in the severity of the movement disorder presumably was related to the effects of carboplatin on a brain already compromised by FXTAS. There was no follow-up on her cognitive status, but the report of both this woman and her husband suggests that cognition also seemed to have improved somewhat following discontinuation of carboplatin.

A similar difficulty in attributing causation was present in the report by Greco et al. (2008), who discussed the clinical history, physical examination, and neuropathologic findings of a woman with signs of FXTAS in conjunction with a diagnosis of relapsing-remitting multiple sclerosis (MS) beginning at age 32. Her condition deteriorated over time, with pathologic and neurologic signs of both neurologic disorders, and she died at 52. She demonstrated deficits in circumscribed areas of cognitive functioning, especially remarkable for disinhibition, problems in working memory, and depression. Because this pattern of impairment is consistent with both FXTAS and MS (disinhibition and working memory deficits both being associated with EF), it was not possible to attribute the deficits, or even many of the autopsy findings, to one disease or the other. At most one can conclude that both disease processes are likely to have played a role in shaping this patient's neuropsychological disorder, and possibly accelerating its progression.

Case reports of women with FXTAS and dementia include a 72-year-old woman with 103 CGG repeats, presenting with tremor, premature ovarian failure, cognitive impairment, and radiologic signs (Al-Hinti et al. 2007). Karmon and Gadoth (2008) discussed a 62-year-old woman with 75 CGG repeats, MRI findings consistent with FXTAS, and progressive cognitive decline. Yachnis et al. (2010) described a 58-year-old woman with parkinsonism, progressive dementia (including bradyphrenia, impaired memory, executive dysfunction, and nonfluent speech), and extensive MRI white matter changes. This patient had neither intention tremor nor ataxia, but met FXTAS diagnostic criteria (Tassone et al. 2012a). Rodriguez-Revenga et al. (2010) described two mother–daughter pairs; all four women had FXTAS, and both mothers had dementia. Interestingly, the daughters had higher CGG repeat sizes (156 and 134, respectively) than their mothers (98 and 88, respectively).

The largest case series of female carriers to date, reported by (Tassone et al. 2012a), emphasized clinical, molecular, and neuropathological findings. Of the eight premutation women discussed, four had dementia (three of whom also had FXTAS). On postmortem examination, three of the four women with dementia had pathological changes consistent with Alzheimer's disease (amyloid plaques and neurofibrillary tangles), and a fourth had cortical Lewy bodies. All women had the ubiquitin-positive intranuclear inclusions associated with FXTAS, even though not all of them had been clinically diagnosed with FXTAS. This study shows that intranuclear inclusions may be present before clinical signs become apparent. It seems

likely that at a minimum, parallel pathophysiological processes would necessarily precede the development of clinical FXTAS.

Only four case reports and two case series of women carriers with dementia have been published to date (Al-Hinti et al. 2007; Karmon and Gadoth 2008; Rodriguez-Revenga et al. 2010; Tassone et al. 2012a; Yachnis et al. 2010), and some of these may have had cognitive impairment associated with other pathology. The findings of Tassone et al. (2012a), for example, suggest that comorbid Alzheimer's disease could have contributed to or accounted for the dementia observed in three women with FXTAS.

Neuropsychological Phenotype of Asymptomatic Female Carriers

Although there are no comprehensive studies of the neuropsychological phenotype of women with the premutation, there have been several studies that focused on relatively circumscribed aspects of cognition. For example, female carriers have been found to have difficulties with mathematical skills and with attention (Hunter et al. 2010, 2012; Lachiewicz et al. 2006; Semenza et al. 2012; Wadell et al. 2013). In one study, among 39 women with the premutation, only nine scored at or above the 50th percentile on the arithmetic part of a standardized achievement test, yet half scored at least at the 50th percentile on the reading and spelling subtests, showing that mathematical skills are an area of relative weakness (Lachiewicz et al. 2006). This is one respect in which women with the premutation are similar to females with a full mutation (Grigsby et al. 1990).

Goodrich-Hunsaker et al. (2011) revealed subtle, yet significant age- and CGG repeat size-related cognitive impairments in female carriers 21–42 years old. Female carriers had a shorter reaction time on a quantitative magnitude comparison (distance effect) task compared with age-matched women with a normal *FMR1* allele. Performance was inversely associated with increasing CGG repeat size (from 67 to 150) and with age although the relationship with age was not statistically significant (Goodrich-Hunsaker et al. 2011).

Sterling et al. (2013) studied the recorded spontaneous 5-min speech samples obtained from a group of 193 women with the premutation, and compared them with samples from 170 women who had a normal FMR1 allele. The authors found a greater number of language dysfluencies that might be associated with deficits in organization and planning among the carriers. This grew worse with age for the carriers, but not the controls. Dysfluencies include such interruptions of speech as incomplete utterances and "filled pauses" (i.e., lexical units with no specific meaning, such as um, uh, ah, and so on). The speech of carriers was marked by significantly more language dysfluencies than was true of those with might be an early indicator of cognitive aging.

Kraan et al. (2013) used a dual-task protocol to assess certain aspects of functioning among female premutation carriers. Dual-task measures are especially

sensitive to subtly compromised functioning or limited processing resources, and the effects are typically most noticeable when both tasks involve the same functional system (e.g., movement). In one study, they examined visual contrast sensitivity, reaction time, proprioception, muscle force, and postural sway. On the measure of postural stability, for example, participants first stood on the floor, and then on a 15 cm thick foam pad, with the eyes open and then closed. The sway meter they used measured anterior–posterior and medial–lateral movement at the waist. In the dual-task version, participants were scored for their performance on a verbal fluency EF task while seated, or while standing on the floor or the foam pad using the sway meter. Those with the premutation performed more poorly than controls on the verbal fluency task, motor reaction time, and a measure of proprioception. They also showed greater instability on the test of postural sway, during which they also fared less well on the verbal fluency task.

In a second paper (Kraan et al. 2014), these researchers studied the effects of a cognitive task on gait in the same sample, again using a dual-task approach. Subjects walked an approximately 6-m course with embedded pressure sensors, at their preferred speed under three conditions: (1) walking only (the baseline measure); (2) walking while counting backward aloud by 3 s; and (3) walking while counting backward aloud by 7 s. Measurements of gait included velocity, stride length, and step time variability, while the number of correct subtractions on dual-task items was used as a measure of cognition on this task. The letter-number sequencing subtest of the WAIS-IV was used as a baseline measure of working memory. There were no between-group differences on baseline measures of either working memory or gait. Those women with the premutation performed less well than those with normal alleles on the dual task items, demonstrating the effects of cognitive load on motor functioning among carriers, but not controls. These authors also showed that the interaction between age and CGG repeat size was strongly predictive of gait variability during dual-task performance. Thus, just as Cornish and her colleagues found with males (Cornish et al. 2011), research is emerging that highlights an interaction of age and CGG repeat length with cognitive or neuromotor variables in women with the premutation.

These findings suggest a possible at-risk phenotype for cognitive impairment in female carriers, or perhaps subtle cognitive deficits that may be precursors of FXTAS in a subset of persons with the premutation. They also suggest the utility of the dual-task approach for detecting subtle differences in functioning between groups, especially when subtly compromised functioning leads to recruitment of executive ability in order to permit normal performance of a task that otherwise might be performed correctly and automatically, with little or no deliberate conscious effort.

A third study by the same group analyzed motor reaction time in both single- and dual-task conditions involving moving one or the other foot in response to a visual cue and a semantic verbal fluency task (Hocking et al. 2015). Again, increasing cognitive load on subjects affected motor performance more for those with the premutation than those without it. In this study, *FMR1* mRNA levels were positively correlated with increasing CGG repeat size. When the PM group was split into

high-repeat (>81 CGG repeats) and low-repeat (≤81 CGG repeats) groups, dual-task effects on reaction time and intra-individual variability in reaction time were both significantly greater in the high-repeat group than the low-repeat group.

In summary, our knowledge of cognition among female premutation carriers—both who do and do not develop FXTAS—is limited. The data that exist suggest that the pattern of deficits may be similar to that observed among males, but this remains to be demonstrated. Adams et al. (2007) found whole-brain white matter hyperintensity (WMH) volume was greater among women with FXTAS than among asymptomatic female carriers or controls only at age 70 and above (Adams et al. 2007). Given that the volume of WMH is positively correlated with decline in EF, regardless of location in the brain (Kramer et al. 2007; Tullberg et al. 2004), it is reasonable to hypothesize that executive deficits may affect women with FXTAS somewhat later in the course of the disorder than is the case for men.

Shelton et al. (2015) studied the effects of increased cognitive load in 14 female carriers of the premutation and 13 age- and IQ-matched controls, using an *n*-back test that measured eye movements as responses. The carriers were unaffected by increases in cognitive load during the working memory task based on their error rate although they were slower to respond than was the case for controls. The meaning of the results, of course, depends on the integrity of eye movement among the carriers, something that has not been established.

As noted previously, several case reports of FXTAS concerned women with comorbid neurologic disorders (Alzheimer's disease, multiple sclerosis) or other conditions that might affect cognition (cancer chemotherapy). A study by (Coffey et al. 2008) strongly suggests that the range of comorbid medical disorders for which female premutation carriers are at risk, and that may preferentially affect those women who have FXTAS, is broad in comparison with males. Female carriers have a higher than expected prevalence of several autoimmune and inflammatory diseases, including hypothyroidism and fibromyalgia. Especially striking is the prevalence of autoimmune thyroid disease among women who also have FXTAS (50 %). Hypothyroidism may cause mood, anxiety, and cognitive disorders, and is a significant risk factor for cognitive impairment (Grigorova and Sherwin 2012). Chronic systemic inflammation, which plays an important role in many of these disorders, may adversely affect cognition and mood (Dantzer 2004, 2012). Female carriers with FXTAS also are more likely to be hypertensive, and to have a history of seizures, both of which may contribute to neuropsychological deficits.

The Diagnosis of Dementia

The definition and measurement of *dementia* has not been consistent across studies that have used dementia as an outcome. Few of the clinical studies discussed above have used standard diagnostic criteria such as the National Alzheimer's Coordinating Center criteria for *dementia* (NACC 2006), or the Diagnostic and Statistical Manual of Mental Disorders-4th Edition, Text Revision (DSM IV-TR; APA 2000) criteria

for *dementia* and *cognitive disorder not otherwise specified.* Subtle cognitive deficits will not be picked up by these methods, because by definition, the deficits would have to cause impairment in activities of daily living, in order to meet diagnostic thresholds (APA 2000; NACC 2006).

Two additional problems arise with regard to the application of current diagnostic criteria to FXTAS dementia. First, the DSM IV-TR criteria mandated memory as one of the cognitive domains that must be impaired (APA 2000). However, the main cognitive deficit in FXTAS dementia is a dysexecutive syndrome, whereas memory impairment is not always present (Brega et al. 2008; Grigsby et al. 2008; Seritan et al. 2013a). Second, studies relying exclusively on the MMSE carry a significant risk of false-negative diagnostic assessments, as the MMSE has a low ceiling, examines mostly cortical functions, and does not assess executive functioning. FXTAS dementia combines cortical and subcortical deficits, and the MMSE is incapable of capturing these reliably (Seritan et al. 2008). The DSM-5 (APA 2013) allows a somewhat more flexible diagnosis of neurocognitive disorders (NCDs) because for the most part memory impairment is no longer a prerequisite, and deficits in only one cognitive domain are considered sufficient for a diagnosis of major NCD, as opposed to two or more domains in the DSM IV-TR.

Similarity of Neuropsychological Findings in FXTAS to Other Disorders

The data concerning cognitive functioning among men with FXTAS are consistent with the pattern of deficits frequently attributed to a class of disorders referred to as mixed cortical-subcortical dementias, or white matter dementias (Filley et al. 2014; Schmahmann et al. 2008). Because the neuropathology of FXTAS is not localized, but affects many regions of the brain (Brunberg et al. 2002; Greco et al. 2002, 2006, 2007), we use the functional term *dysexecutive syndrome* to refer to the pattern of deficits associated with FXTAS, rather than the more anatomical terms *frontal*, *cortical*, or *subcortical*, which suggest focal or more circumscribed pathology.

Cognition is impaired to varying degrees in a number of neurodegenerative movement disorders, and other comorbid psychiatric symptoms also may be prominent features of these syndromes, as is the case in Lewy Body disease and Huntington's disease (HD). Ordinarily, the types of cognitive deficits observed in movement disorders differ significantly from the dementia that characterizes AD. This is clearly the case in FXTAS.

The typical features of these disorders include deficits in EF (frequently presenting as personality change), slowed information processing, defective working memory, and impaired declarative memory. Such disorders include Huntington disease, Parkinson disease, progressive supranuclear palsy (PSP), multiple sclerosis, several of the spinocerebellar ataxias (SCAs), corticobasal degeneration (CBD), the leukodystrophies, and many others. Although the clinical presentation may differ somewhat in its specific features across syndromes, they are more similar to one

another than to the cortical dementia observed in AD (Bak et al. 2005; Burk et al. 2003; Dubois and Pillon 1998; Herman-Bert et al. 2000; Kertesz et al. 2000, 2005; Liu et al. 2004; McKhann et al. 2001; O'Hearn et al. 2001; Schelhaas and Van De Warrenburg 2005; Schmahmann et al. 2008; Vuillaume et al. 2002).

The cognitive deficits associated with FXTAS appear most similar to those observed in several of the spinocerebellar ataxias (SCA) and in some subtypes of frontotemporal dementia (FTD). For example, individuals with SCAs 1, 2, 3, 6, 7, and 19 have been reported to show deficits in executive functioning and information processing, sometimes in association with other cognitive problems. Some of these disorders, however, such as SCA 13, may be associated with more significant global cognitive impairment. Frontotemporal dementia (FTD) is a diverse group of degenerative disorders that may have different manifestations, with both frontal (*fv*) and temporal (*tv*) variants. Related disorders, which may affect EF, include corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and FTD with parkinsonism (FTDP-17), among others. There are, however, important differences among these disorders. For example, *tv*FTD is characterized by aphasia, while the movement disorder observed in others differs significantly from FXTAS (e.g., alien hand, apraxia, and reflex myoclonus in CBD; gaze palsy and neck dystonia in PSP).

Seritan et al. (2008) studied the relationship of the cognitive deficits observed in FXTAS with those of Alzheimer's disease. Beginning with a chart review of 50 men and 18 women with FXTAS, 20 males were selected who met the National Alzheimer's Coordinating Center criteria for dementia (i.e., impairment in two or more cognitive domains, progression of deficits, and functional decline secondary to the cognitive dysfunction). These individuals were matched with 70 persons with mild AD on age, sex, education, and stage of dementia (mild, MMSE > 24).

The mean age of men with FXTAS who met criteria for dementia or other cognitive impairment ($n = 50$) was 69.2 years, ranging from 55 to 89. Men with FXTAS who were classified as demented were significantly older than the overall group of subjects with FXTAS, as might be expected given longer duration of a progressive neurodegenerative disorder. In the subgroup of cognitively affected men with FXTAS, motor symptoms generally were reported to precede the onset of cognitive difficulties by a variable interval, between 1 and 13 years (mean time interval of 3.7 years). One subject was an exception, presenting with cognitive and behavioral problems by approximately 2 years before the onset of clinically significant tremor and ataxia. Given that these data were obtained from a chart review, however, and that early indications of dysexecutive syndrome may be very subtle, we can't draw solid conclusions regarding the relative onset of cognitive and movement disorders.

Performance on a measure of verbal fluency used to assess EF (Controlled Oral Word Association Test) was lower among subjects with FXTAS dementia (mean = 22.8) than patients with AD (mean = 28.8) although the difference was not statistically significant due to the small sample and intra-group variability (the difference, however, is mildly clinically meaningful). One working memory measure (Digit Span backward raw score, 4.8 in AD vs. 5.4 in FXTAS), as well as the Boston

Naming Test (48.5 in AD vs. 54.2 in FXTAS), also were not significantly different. The only significant difference was noted on the Digit Span forward raw score (measuring attention), which was higher among subjects with FXTAS dementia (8.6 vs. 7.1 in AD, $p=0.01$).

Comparisons of AD and FXTAS should be replicated and elaborated before they can be generalized. In particular, a more thorough assessment of different aspects of cognition would be of some value. For example, neither visuospatial ability nor declarative learning—both of which demonstrate significant impairment early in the course of AD—was examined in this study. However, several differences between the two disorders were apparent. Naming was marginally impaired in AD relative to FXTAS, as might be expected given that AD, but not FXTAS, is associated with significant language impairment over time. For most individuals with FXTAS, both speech and language are intact, although female premutation carriers may show the language dysfluencies observed by Sterling et al. (2013), while individuals with advanced FXTAS may show a cerebellar type of dysarthria (Grigsby et al. 2006b). On the other hand, persons with FXTAS appear to perform more poorly on measures of executive function, such as verbal fluency, consistent with its primarily dysexecutive presentation.

Noncognitive Psychiatric Disorders in FXTAS

Although the findings are somewhat mixed, most previous research has shown that adult carriers of the premutation are likely to experience significant psychiatric symptoms, irrespective of whether they have FXTAS. Some authors found no support for a higher rate of psychiatric dysfunction among female carriers (Reiss et al. 1993; Sobesky et al. 1994b); others have obtained results indicating an increased prevalence of interpersonal sensitivity, depression (Johnston et al. 2001), obsessive-compulsive symptoms (Hessl et al. 2005), and schizotypal features, as well as disorders characteristic of the schizophrenic spectrum (Freund et al. 1992; Sobesky et al. 1994a). More recent studies yielded strong evidence for an increased prevalence of anxiety and depressive symptoms among female carriers (Bourgeois et al. 2011; Obadia et al. 2013; Roberts et al. 2009; Rodriguez-Revenga et al. 2008, 2010; Seritan et al. 2013b, c). The strength of these studies lies in the fact that the authors used standardized instruments for the diagnosis of psychiatric conditions, such as the Symptom Checklist-90-Revised (SCL-90-R; Hessl et al. 2005) and the Structured Clinical Interview for DSM IV-TR (SCID; Bourgeois et al. 2011; Roberts et al. 2009; Seritan et al. 2013b, c).

Research on male premutation carriers suggests a higher rate of anxiety (Jacquemont et al. 2004a) and obsessive-compulsive symptoms (Dorn et al. 1994; Hessl et al. 2005). Cornish and her associates found an increased prevalence of problems in social cognition among those with the premutation (Cornish et al. 2005). Among men with FXTAS, a number of psychiatric symptoms have been reported (e.g., Bourgeois et al. 2007; Bourgeois et al. 2011; Hagerman et al. 2001;

Jacquemont et al. 2004a). Additionally, both male and female carriers have been found to have symptoms, or meet full criteria for, alcohol and drug use, autism spectrum disorders, bipolar disorder, and psychosis, as well as eating, impulse control, and somatoform disorders (Bailey et al. 2008; Harris et al. 2008; Hatton et al. 2006; Kogan et al. 2008; Rogers et al. 2001; Seritan et al. 2009, 2013c). The findings are discussed in more detail below.

It appears that behavioral disturbances, with disinhibited and socially inappropriate behavior, mood lability, irritability, and agitation, are prominent in FXTAS. These are consistent with the dysexecutive syndrome identified in neuropsychological studies. Bacalman et al. (2006) studied 14 males with FXTAS and 14 age- and education-matched controls, using the Neuropsychiatric Inventory (NPI; Cummings et al. 1994). The NPI is a structured caregiver interview used to delineate 12 domains of neurobehavioral symptoms co-occurring in dementing disorders: delusions, hallucinations, agitation, depression, anxiety, elation, apathy, disinhibition, irritability, aberrant motor behavior, and sleep and appetite changes. Bacalman and her colleagues did not establish clinical diagnoses of dementia although MMSE scores ranged from 16 to 30. Only three subjects with FXTAS had MMSE scores <24, suggesting dementia; the remainder had scores ≥26. 57 % of men with FXTAS, and no controls, showed evidence of agitation or aggression; 79 % (and 14 % of controls) were depressed; 93 % (and no controls) showed signs of apathy; 64 % (and no controls) were disinhibited; and 86 % manifested irritability (compared to 7 % of controls). The severity of neurobehavioral impairments did not correlate with MMSE scores. In addition, Seritan and colleagues reported that 60 % of patients with FXTAS dementia presented with personality changes, as identified by family caregivers (Seritan et al. 2008).

Bourgeois et al. (2007) reported a series of 15 cases of males with FXTAS. The mean age was 64 years, with median and mean time since onset of 7 years and a mean of 84 CGG repeats. All subjects were administered a brief neuropsychological examination, clinical neurological and psychiatric exams, and the NPI. Six cases were diagnosed with mood disorders; three of these, and one additional individual, were diagnosed with anxiety disorders. Seven patients were described as apathetic, a component of the dysexecutive syndrome.

Hessl et al. (2005) used the Symptom Checklist-90-R (SCL-90-R) to study a relatively large sample of both male and female carriers of the premutation (144 women and 68 men). Among these individuals, 42 males (62 %) and 22 females (15 %) had a diagnosis of FXTAS. Men with FXTAS scored higher than the asymptomatic carriers on measures of somatization, interpersonal sensitivity, depression, anxiety, phobic anxiety, and psychoticism. Somewhat similar findings were observed for females with FXTAS, with elevations on somatization, obsessive-compulsive symptoms, depression, and overall symptom severity. Both male and female carriers without FXTAS had elevations on the obsessive-compulsive scale, and men showed increased overall symptom severity. The SCL also provides ratings of psychiatric "caseness." Hessl et al. (2005) found that for males and females with FXTAS, the percentages of subjects meeting criteria for caseness were 52.4 and 45.5 %, respectively, while for male and female carriers the corresponding rates were 38.5 and 22.1 %.

Bourgeois et al. (2011) assessed lifetime history of mood and anxiety disorders in fragile X premutation carriers (both with and without FXTAS) using the Structured Clinical Interview for DSM-IV (SCID). The authors compared participants' reported lifetime history of mood and anxiety disorders to age-specific population estimates from the National Comorbidity Survey Replication (NCS-R). Sixty-five percent ($N=30$) of the subjects with FXTAS had a lifetime history of a mood disorder and 52 % (24) had a lifetime history of an anxiety disorder, which was statistically significantly higher than age-specific population estimates. Suicidal thinking was present in about 5 % of participants seen at one research center, highlighting the need for careful risk assessments (Seritan et al. 2013c). Seritan et al. (2013b) studied 81 premutation carriers (34 men, average age 62 years) with and without FXTAS, and compared their reported age of onset for mood and anxiety disorders to the corresponding onset ages in the general population. The median ages of onset for major depressive disorder (MDD) (46 years old, $p<0.0001$), panic disorder (40 years old, $p=0.0067$), and specific phobia (11.5 years old, $p=0.0003$) were significantly higher in premutation carriers than in the general population. Median MDD age of onset among male carriers was 52 years old, and for those with FXTAS it was 49.5 years; these were significantly higher relative to the general population (median = 32). The average age of onset for tremor and ataxia was significantly later than for MDD and anxiety disorder. This was an interesting finding, suggesting that psychiatric symptoms, especially mood and anxiety disorders, may precede tremor and ataxia in FXTAS.

Summary

A consistent pattern of neuropsychological/neuropsychiatric deficits has been observed in FXTAS. The primary cognitive impairment observed among both males and females with FXTAS seems to be a dysexecutive syndrome although there may be somewhat more variability in the female phenotype. Defective executive functioning appears to affect both declarative and procedural learning adversely, as well as performance on a range of complex tasks. In contrast to the dementia associated with Alzheimer disease, mental status and general intellectual functioning (e.g., as measured by IQ) typically remain in the average range until the late stages of FXTAS. The exceptions to this generalization are observed in subtests and items that are dependent on the executive abilities and working memory. Because of the loss of the capacity for behavioral self-regulation, changes in personality (lack of insight, disinhibition, and failure to initiate goal-directed behavior) are especially noteworthy. Other neuropsychiatric features of FXTAS include disturbances of mood, anxiety, somatization, irritability, agitation, and obsessive-compulsive symptoms.

Data analyzed to date have been cross-sectional, and therefore it is not yet possible to draw inferences regarding the natural history of cognitive decline in FXTAS. The individual histories of participants in the FXTAS group suggest that

the cognitive disorder is progressive, but the rate and nature of decline remain to be determined. Moreover, as some participants with FXTAS obtained scores suggestive of minimal or no impairment, it is not clear that all individuals affected by FXTAS will experience significant cognitive problems. When cognition is affected, the pattern of deficits may vary somewhat across individuals. Further research will enhance our understanding of this disorder, of the temporal and symptomatic relationships between neuropsychological and neurological findings, and of other genetic, epigenetic, and environmental variables that determine the development, course, penetrance, and severity of FXTAS.

Both males and females with FXTAS have a high rate of noncognitive psychiatric disorders, which often antedate the development of the movement disorder. Their temporal relationship with the cognitive features of FXTAS is unclear. Often these mood and anxiety episodes are identified in retrospect, especially if the patient did not seek clinical psychiatric care for them. Clinical vigilance for mood and anxiety syndromes in known fragile X premutation carriers (even in advance of the development of the movement and neurocognitive disorders characteristic of FXTAS) is warranted, and clinical intervention may be beneficial. More importantly, inasmuch as these episodes may represent a psychiatric prodrome of later FXTAS, attention to the possible development of movement and neurocognitive disorders in middle aged (especially male) premutation carriers with mood and anxiety disorders could lead to disease-modifying treatment of the FXTAS patient's condition at an earlier stage of neuropsychiatric impairment.

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Chapter 4
Neuroimaging Findings in FXTAS

Emily S. Halket, Jun Yi Wang, David Hessl, and Susan M. Rivera

Abstract In this chapter, we review the neuroimaging findings associated with fragile X-associated tremor/ataxia syndrome (FXTAS). We review what is currently known about radiological signs of the disorder including both white matter lesions and mild to severe cortical loss. We also review findings on the integrity of white matter tracts in the brain (using diffusion tensor imaging) and in vivo brain function (using functional magnetic resonance imaging) in individuals with the FX premutation with and without FXTAS. Brain-molecular relationships in FXTAS including associations that have been observed between cortical and white matter changes and molecular variables such as CGG repeat length are also discussed. Similarly, we review relationships that have been reported between brain abnormalities and the neuropsychological phenotype in FXTAS. While much knowledge has been gained about the neuropathology of FXTAS, future work will focus on continuing our understanding of the developmental time course of this pathogenesis as well as searching for brain biomarkers of the disease in asymptomatic premutation carriers.

Keywords FMR1 gene • Fragile X premutation • White matter disease • Neurodegenerative disease

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Introduction

Pathological hallmarks of FXTAS, observed on autopsy, include ubiquitin-positive intranuclear inclusions in neurons and astrocytes throughout the cortex and brainstem, as well as axonal degeneration in cerebellar Purkinje cells (Arocena et al. 2005; Greco et al. 2006; Greco et al. 2002; Iwahashi et al. 2006; Louis et al. 2006), and abnormal white matter with axonal degeneration, spongiosis, and myelin loss (see Chap. 5). The chapter will focus on features of the FXTAS brain that correspond to these neuropathological changes and can be seen on examination of magnetic resonance imaging (MRI) images. These radiological signs of FXTAS include MRI white matter lesions involving the middle cerebellar peduncles (the so-called MCP sign), moderate to severe generalized brain atrophy, and MRI lesions involving cerebral white matter. This chapter will also review what is currently known about structural (morphometric), functional, and white matter microstructural differences in the brains of those with FXTAS (See Table 4.1 for summarized findings).

Radiological Findings in FXTAS

The MCP Sign

The radiological feature considered to be the most distinct sign of FXTAS is the presence of bilateral regions of increased T2 signal intensity in the middle cerebellar peduncles (MCPs) and in underlying cerebellar white matter lateral to the dentate nuclei on T2-weighted or FLAIR MR imaging. In the first study of the MR characteristics of this disorder, Brunberg et al. (2002) found that 15 of 17 patients examined showed symmetrically decreased T1 and increased T2 signal intensity in cerebellar white matter, and 14 of the 17 had similar signal intensity alterations of the MCPs. The "MCP sign" is thus a primary diagnostic criterion for FXTAS, and the neuropathological basis of this increased signal is thought to be spongiosis of the deep cerebellar white matter (see Fig. 4.1). The MCP sign is not specific to FXTAS. It has in fact been described in a number of diseases, including multiple system atrophy (MSA) (Ngai et al. 2006; Storey and Billimoria 2005). Likewise, not all patients with FXTAS demonstrate this finding (Adams et al. 2007; Jacquemont 2005; Jacquemont et al. 2004), and it is estimated to occur in approximately 60 % of males and 13 % of females with FXTAS (Adams et al. 2007). The MCP sign has also been reported in the absence of neurological symptoms of FXTAS in two relatively young (aged 52 and 39 years) male premutation carriers (Loesch et al. 2008) and in a 73-year-old asymptomatic premutation carrier (Capelli et al. 2007) suggesting that abnormal MCP signal may predate symptoms and be an early sign of impending clinical disease.

Table 4.1 Summary of neuroimaging findings associated with FXTAS, as discussed in text

Study	Participants	Imaging methodology	FXTAS-relevant findings
Adams et al. (2007)	20 PC women without FXTAS, 15 PC women with FXTAS, 11 control women, 25 PC men without FXTAS, 36 PC men with FXTAS, 39 control men	Conventional and Volumetric MRI	• Women with FXTAS: reduced brain volume, reduced cerebellar volume, and increased WM disease compared
			• Volume reductions to lesser extent in women than men with FXTAS
			• MCP sign in 13 % of women
Brunberg et al. (2002)	17 PC men with signs of FXTAS and 14 control men	Conventional MRI	• 15/17 increased T2 signal intensity in cerebellar WM
			• 14/17 MCP sign
			• 16/17 cerebellar atrophy and all with cerebral atrophy
			• 14/17 thinning of corpus callosum
Cohen et al. (2006)	11 PC men without FXTAS, 25 PC men with FXTAS, 21 control men	Conventional and Volumetric MRI	• Decreased volume in cerebrum, cerebellum, brainstem
			• Enlarged ventricles
			• Increased whole brain and cerebellar WM hyperintensity
Hashimoto et al. (2011a)	15 PCs (8 men) without FXTAS, 15 PCs (6 men) with FXTAS, and 12 controls (7 men)	fMRI	• PCs with FXTAS showed reduced activation in right ventral IFC and left dorsal IFC/PMC during working memory task
Hashimoto et al. (2011b)	24 PC men without FXTAS, 31 PC men with FXTAS, 28 control men	Conventional and Volumetric MRI	• Cortical and subcortical grey matter loss
Hashimoto et al. (2011c)	35 PC men with FXTAS, 16 PC men without FXTAS, 20 control men	DTI	• Reduced FA in MCP, SCP, cerebral peduncle, fornix, and stria terminalis
Jacquemont et al. (2003)	20 PC men with FXTAS and 20 control men	Conventional and Volumetric MRI	• 75 % with decreased cerebral cortical volume
Renaud et al. (2014)	22 PCs (17 men) with signs of FXTAS and 125 neurological patients (68 men) with normal *FMR1* allele	Conventional MRI	• MCP sign in 14/17 men and 0/5 women with suspected FXTAS
			• Hyperintensity of the splenium in 11/17 men and 3/5 women with suspected FXTAS.
			• Hyperintensity of the brainstem in 9/22 men and women with suspected FXTAS

(continued)

Table 4.1 (continued)

Study	Participants	Imaging methodology	FXTAS-relevant findings
Wang et al. (2012)	26 PC men without FXTAS, 15 PC men with FXTAS, 34 control men	DTI	• Reduced structural connectivity in motor, limbic, association, and callosal fiber tracts
			• Increased age-related decline in structural connectivity in limbic, association, and callosal fiber tracts
Wang et al. (2013a)	11 PC men without FXTAS, 36 PC men with FXTAS, and 14 control men	Conventional and Volumetric MRI	• Volume loss in bilateral thalamus and putamen, left caudate, and right pallidus
Wang et al. (2013b)	26 PC men without FXTAS, 36 PC men with FXTAS, 34 control men	DTI	• Reduced tract volume in descending motor tract, MCP, SCP, and anterior corpus callosum
			• Elevated MD in MCP, SCP, and anterior corpus callosum
			• Reduced FA in SCP and elevated FA in descending motor tract

PC premutation carrier, *WM* white matter, *FA* fractional anisotropy, *MCP* middle cerebellar peduncle, *SCP* superior cerebellar peduncle, *IFC* inferior frontal cortex, *PMC* premotor cortex

Abnormal Cortical White Matter Signal

FXTAS-associated MRI findings also include patchy or confluent areas of increased signal intensity on T2-weighted or FLAIR images in periventricular and deep white matter of the cerebral hemispheres and corpus callosum. These cerebral alterations are more prominent than in age-matched controls (Brunberg et al. 2002; Cohen et al. 2006) although they are somewhat nonspecific as they can be seen in many common conditions in the population, such as hypertension, vascular disease, and even normal aging. In addition to the MCP sign, a recent examination of white matter in aging premutation carriers with symptoms of FXTAS suggests that increased signal intensity in the splenium is also a marker of FXTAS disease (Renaud et al. 2014). In this study, frequency of hyperintensity in the splenium was comparable to frequency of MCP sign in those suspected to have FXTAS. While, as expected, hyperintensity in the MCP was more frequent in male (14 of 17) than female (0 of 5) patients, hyperintensity in the splenium was observed at roughly equivalent frequencies in male (11 of 17) and female (3 of 5) patients.

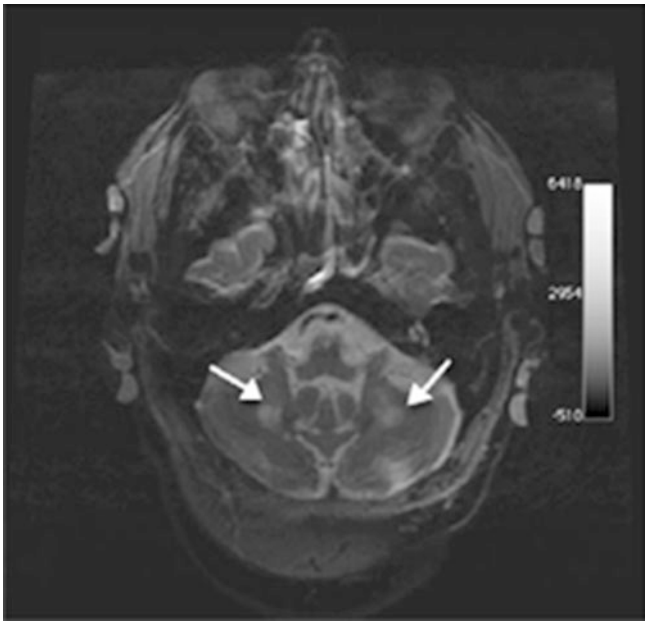

Fig. 4.1 T2-weighted axial image of the brain of a 65 y.o. FXTAS patient with 85 CGG repeats, 15 years of education, and tremor onset at 62 years of age. *Arrows* point to probable spongiform changes in the middle cerebellar peduncles (the "MCP sign"), which appear as white matter hyperintensities. (NINDS grant number NS044299 [JG])

Cerebral Atrophy

Individuals with FXTAS also present with significant cerebellar and cerebral atrophy. Early studies showed cerebellar cortical atrophy in as many as 16 of 17 and general cerebral atrophy in virtually all patients studied (Brunberg et al. 2002; Greco et al. 2006; Greco et al. 2002; Jacquemont et al. 2003; Leehey et al. 2003). Individuals with FXTAS also exhibited significantly enlarged ventricle size (as measured by Evan's Index) as compared to the age-matched controls, and 14 patients showed thinning of the corpus callosum (Brunberg et al. 2002). Middle cerebellar peduncles were also significantly smaller in the FXTAS group than in the control group.

Subsequent morphometric studies performed in carriers with and without FXTAS support these initial findings, revealing significant volume loss in the cerebellum, cerebral cortex, amygdalo-hippocampal complex, thalamus, basal ganglia, and brainstem, as well as increased ventricle size (Cohen et al. 2006; Hashimoto et al. 2011b; Loesch et al. 2005; Moore et al. 2004; Wang et al. 2013a). Throughout the cerebellum, patients with FXTAS show significant gray matter loss compared to healthy controls (Hashimoto et al. 2011b). When compared to premutation carriers

without FXTAS, those with FXTAS show increased cerebellar atrophy in vermis lobules IV/V, VI, and VII and hemispheric lobules IV/V, VI, Crus 1, and right Crus II. Premutation carriers without FXTAS also show decreased cerebellar volume compared to healthy controls in vermis lobule I/II and lobule III in the left hemisphere. A separate study reported decreased volume in the anterior cerebellum (lobule VI) in carriers without FXTAS (Battistella et al. 2013). Thus, premutation carriers with and without FXTAS present with decreased cerebellar grey matter volume; however, the presentation is to a lesser extent in carriers without FXTAS. Areas of the cerebellum that do show grey matter atrophy in carriers without FXTAS, particularly the anterior vermis, may provide precursory evidence of FXTAS.

Within the cerebrum, premutation carriers with FXTAS show prominent loss compared to healthy controls in dorsomedial frontal and parietal regions, insula, medial temporal regions, and lateral prefrontal regions (Hashimoto et al. 2011b). When compared to premutation carriers without FXTAS, individuals with FXTAS show grey matter loss in dorsomedial PFC and the precuneus. In this analysis, premutation carriers without FXTAS did not show significant grey matter loss in the cerebral cortex compared to healthy controls. A study examining subcortical grey matter volume in premutation carriers reported significant grey matter loss in thalamus and basal ganglia in FXTAS (see Fig. 4.2) (Wang et al. 2013a). Compared to healthy controls, individuals with FXTAS show grey matter loss in thalamus, putamen, caudate, and globus pallidus. Compared to carriers without FXTAS, those affected by FXTAS showed greater atrophy in bilateral thalamus and putamen. Further, atrophy in bilateral thalamus, putamen, and left caudate correlated with increasing FXTAS stage (using the FXTAS clinical staging scale, see Chap. 1). This analysis did not reveal significant subcortical atrophy in carriers without FXTAS compared to healthy controls.

Many of the radiological features of FXTAS correlate with CGG repeat length in males. Significant associations have been observed between reduced cerebral and cerebellar volume and increased length of the CGG repeat expansion in male premutation carriers, but not in females (Adams et al. 2007). Within the cerebrum, increased CGG repeat size is correlated with decreased volume in supplementary motor area and dorsomedial prefrontal cortex (Hashimoto et al. 2011b). CGG repeat length is also correlated with increased ventricular volume and whole-brain white matter hyperintensity (Cohen et al. 2006).

Findings in White Matter Structural Integrity

As discussed above, MRI findings in individuals with FXTAS often include abnormal high T2 signal consistent with abnormalities in periventricular and subcortical cerebral white matter, as well as characteristic high T2 signal lesions in the middle cerebellar peduncle (MCP sign), which can also be seen in the cerebellar white matter (Cohen et al. 2006). Although such findings are common in more advanced FXTAS cases, standard T2-weighted MRI scans may not be sensitive to white

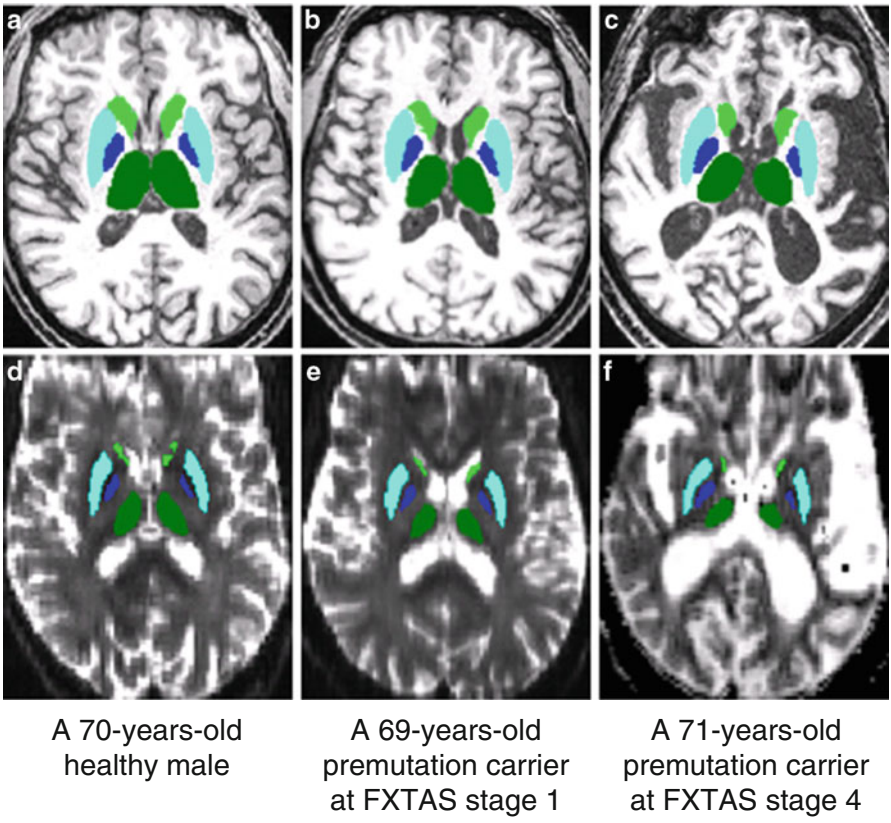

Fig. 4.2 Segmentation of subcortical gray matter. (**a–c**) T1-weighted images. (**d–f**) MD maps. *Green*, thalamus; *light green*, caudate nucleus; *light blue*, putamen; *blue*, globus pallidus

matter changes when the individual first becomes clinically symptomatic, or even prior to symptom onset. Alterations in MRI T2 signal are due to increased brain interstitial water content, and multiple MRI techniques are available to measure changes in water content. One technique that takes advantage of this hydrogen-based alteration in signal is diffusion tensor imaging (DTI). This technique makes it possible to examine alterations in the microstructure of white matter in vivo.

DTI is based on the application of diffusion-weighted gradients and measuring the direction and magnitude of hydrogen movement (Basser et al. 1994). The three-dimensional geometry of the diffusion in a particular volume element (voxel) can be described by a "tensor" (Basser and Pierpaoli 1996). From the diffusion tensor in each voxel, one can derive three eigenvalues (λ_1, λ_2, and λ_3) defining the magnitude of the diffusion system and the three associated eigenvectors that describe the direction of the diffusion system. The average of the three eigenvalues represents the mean molecular motion (mean diffusivity, MD) that is affected by barriers to diffusion.

Based on the ratio of the three eigenvectors, the intra-voxel directionality of hydrogen diffusion can be determined. This measure is termed fractional anisotropy (FA) and can range from 0 to 1 (Basser and Pierpaoli 1996), with 0 indicating completely random diffusion (isotropic diffusion) and 1 representing completely directional diffusion (anisotropic diffusion). CSF has extremely low FA values because hydrogen is free to diffuse in any direction. Gray matter has low FA because cellular structures (e.g., cell membrane, organelles) impede the free diffusion of hydrogen, but these structures do not promote organized, directional diffusion. Highly organized white matter tracts have high FA because hydrogen diffusion is directionally constrained by the tract's cellular organization.

Two additional diffusion-based measures are commonly used to characterize water diffusion in the white matter: axial diffusivity and radial diffusivity. Axial diffusivity represents the amount of diffusion along the main diffusion direction in a voxel, and radial diffusivity represents the amount of water diffusion perpendicular to the main diffusion direction. DTI can also be used to infer the anatomical connectivity between brain regions. Connecting voxels with similar principal eigenvectors and with FA values above a threshold (to avoid tracking into areas with uncertain directionality), a technique called fiber tracking, infers the trajectories of underlying fiber tracts.

When the barriers to free diffusion of hydrogen in white matter degenerate, such as seen the white matter damage associated with FXTAS, MD increases and the directionality of intra-voxel diffusion becomes more isotropic. Thus, one would predict increased MD and isotropic diffusion (decreased FA) in the white matter of patients with FXTAS because of the degeneration of axons and myelin, increased gliosis, and other axonal changes. These changes are likely to be present some time before the behavioral manifestation of FXTAS, and as such, DTI might provide a sensitive measure of presymptomatic white matter changes.

Commensurate with the finding of increased signal in the MCP on standard T2-weighted MRI scans in premutation carriers affected with FXTAS, DTI results show decreased FA, increased mean diffusivity, and decreased volume in the MCP white matter tracts in male carriers with FXTAS, suggesting a loss of white matter integrity in this region (Hashimoto et al. 2011c; Wang et al. 2013b). In premutation carriers without FXTAS, there is also evidence of increased axial and radial diffusivity in MCP (Battistella et al. 2013; Hashimoto et al. 2011c).

In addition to strong evidence of insult to MCP microstructure in FXTAS, examination of fiber tracts throughout the brain reveals widespread microstructural impairment and decreased connectivity in FXTAS (Hashimoto et al. 2011c; Wang et al. 2012; Wang et al. 2013b). Specifically, white matter degeneration is observed in the SCP, cerebral peduncle, corpus callosum, fornix, stria terminalis, cingulum, extreme capsule, and association fibers such as the arcuate, uncinate, inferior longitudinal, and inferior fronto-occipital fasciculi (see Fig. 4.3).

Structural integrity measures were also found to correlate with genetic measures in male premutation carriers. Diffusivity measures in the MCP correlate with CGG repeat length in an inverted U pattern suggesting a complex relationship between the *FMR1* premutation and white matter degeneration (Hashimoto et al. 2011c).

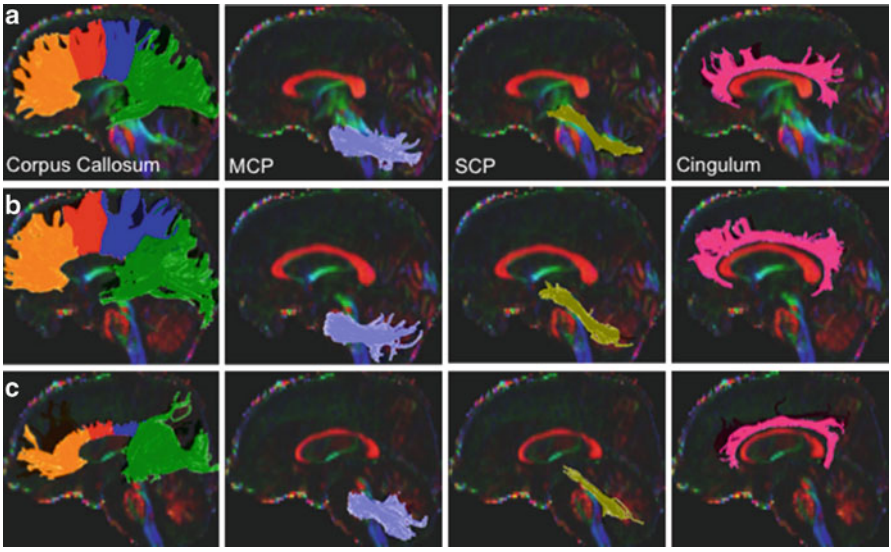

Fig. 4.3 Representative reconstructed fiber tracts (*left-side* only) superimposed on DTI color-coded map. (**a)** a 65-year-old healthy control; (**b)** a 65-year-old premutation carrier without FXTAS (FXTAS stage 0); and (**c**) a 65-year-old premutation carrier at FXTAS stage 4. From *left* to *right* columns: the four regions of the corpus callosum (CC): genu (*orange*), anterior body (*red*), posterior body (*navy blue*), and splenium (*green*); middle cerebellar peduncle (MCP, *purple*); superior cerebellar peduncle (SCP, *gold*); and cingulum (*pink*)

Increased CGG repeat length correlates with decreased structural organization of the SCP, as measured by FA, in patients with FXTAS (Wang et al. 2013b). Additionally, increased repeat length correlates with reduced tract volume in SCP in carriers with and without FXTAS. This study also reported a correlation between heightened levels of *FMR1*-mRNA (as measured in blood) and decreased FA in the SCP in carriers with FXTAS.

The results of these studies demonstrate sensitivity of DTI measures of white matter integrity to the presence of FXTAS symptoms in premutation carriers of the *FMR1* gene. DTI measures detected overall differences in FA between premutation carriers and healthy controls that correlated with the severity FXTAS symptoms and identified specific regions of brain most obviously abnormal including the MCP (Hashimoto et al. 2011c). Evidence of microstructural changes and greater age-related decline, as evidenced in a cross-sectional study, in the corpus callosum suggests involvement of the corpus callosum in the progression of FXTAS (Wang et al. 2012; Wang et al. 2013b). Additionally, DTI connectivity measures implicate the SCP, carrying the majority of efferent fibers from the cerebellum, in FXTAS. Atypical DTI measures seen in non-affected carriers suggest the possibility of presymptomatic alterations in structural connectivity that may identify a group of premutation carriers most at risk of developing FXTAS.

Functional Brain Abnormalities

Functional magnetic resonance imaging (fMRI) is the use of MRI to measure the hemodynamic response related to neural activity in the brain. Neural activity results in an increase in blood flow to the local vasculature and a corresponding local reduction in deoxyhemoglobin. Since deoxyhemoglobin is paramagnetic, it alters the T2* weighted. In this way, with the use of an appropriate MR imaging sequence, human cortical functions can be observed without the use of exogenous contrast enhancing agents. An fMRI study completed in 15 premutation carriers affected by FXTAS, 15 premutation carriers unaffected by FXTAS, and 12 matched healthy controls revealed altered neural activation in carriers with FXTAS. This study examined neural activation during a verbal working memory task and investigated the relationship between cortical activity and molecular variables, including CGG repeat size and *FMR1* mRNA levels (Hashimoto et al. 2011a). Compared with the control group, the premutation carriers affected with FXTAS showed reduced activation in the right inferior frontal cortex, bilateral premotor cortex, and left hippocampus. Because these brain structures are critical for working memory (Cabeza and Nyberg 2000), the findings suggest that functional abnormalities in these areas may be the neural correlates for the deficits in executive function and control of working memory that have been observed in FXTAS patients. The unaffected premutation carriers showed reduced activation only in the right inferior frontal cortex. Regression analysis combining all the three groups revealed significant effects of molecular variables of the *FMR1* gene on activity in several prefrontal areas; in particular, the right inferior frontal cortex showed reduced activation as CGG repeat size and mRNA level increased. The left premotor cortex also showed reduced activation with increasing CGG repeat size. Additionally, a significant correlation was also observed between FXTAS clinical staging scale and brain activity in the right hippocampus. These observations provide converging evidence of compromised brain regions underlying working memory in fragile X premutation carriers. They further demonstrate that areas in the lateral prefrontal cortex are particularly vulnerable to abnormal molecular processes associated with the *FMR1* premutation, which may underlie compromised memory and executive functioning in these individuals. More functional imaging studies are needed, across a number of cognitive domains, to facilitate the creation of a more complete picture of the impact of the fragile X premutation on brain activity in those with and without FXTAS.

Brain–Behavior Relationships

The core behavioral features of FXTAS include progressive intention tremor and cerebellar ataxia (Hagerman and Hagerman 2015). DTI tractography measurements from the corpus callosum and SCP are associated with motor regulation and dexterity in carriers with FXTAS (Wang et al. 2013b). In this study, tractography

measurements in the MCP also explained variability in dexterity performance in carriers with FXTAS. These findings suggest that changes in microstructural integrity of white matter, including the corpus callosum, MCP, and SCP, reflect observable behavioral changes associated with FXTAS. Additionally, an investigation of postural sway in premutation carriers with and without FXTAS provides preliminary evidence of a relationship between cerebellar atrophy and greater postural sway, even when accounting for the effect of age (Birch et al. 2015). This preliminary evidence further supports the implication of the cerebellum in FXTAS and suggests that insult to the cerebellum may be an initial sign of disease-related processes in premutation carriers.

In addition to motoric features of FXTAS, the neuropsychological phenotype of FXTAS includes impairment of specific cognitive abilities, including working memory, executive functioning, and the speed and capacity of information processing (Brega et al. 2008; Grigsby et al. 2008; Grigsby et al. 2007; Grigsby et al. 2006b). Deficits also have been observed on measures of declarative memory, but these appear to be secondary to problems with executive functioning (Brega et al. 2008), rather than primary disorders of memory such as may be seen in Alzheimer's disease.

In the context of the neuroradiologic and neuropathologic findings concerning FXTAS, it is not possible to attribute these cognitive disorders to specific brain loci. Although dysexecutive disorders may be observed in association with lesions of prefrontal cortex (e.g., Fuster 2008), Tullberg et al. (2004) have reported that regardless of their location, higher volumes of white matter hyperintensities are associated with disorders of executive functioning. Thus, white matter hyperintensities found throughout most of the cortex (although apparently sparing the occipital lobes) in FXTAS are likely related to deficits in executive functioning observed in FXTAS. The attempt to define specific relationships between neuroanatomy and neuropsychology in FXTAS is further complicated by the fact that discrete lesions in a number of areas, including the dentate nuclei of the cerebellum, basal ganglia, pons, thalamus, supplementary motor area, and cingulate gyrus may disrupt various aspects of executive functioning (Hallett and Grafman 1997; Leiner et al. 1993; Middleton and Strick 1997; Schmahmann 2000).

Interestingly, the hippocampus has a large burden of inclusion bodies, both in neurons and astrocytes, in comparison with other regions of the brain (Greco et al. 2002, 2006). Nevertheless, morphometric analyses showed no differences between persons with FXTAS, asymptomatic carriers, or controls in hippocampal volumes (Cohen et al. 2006). Despite this lack of morphometric difference, Cohen et al. (2006) also reported that hippocampal volume was significantly associated with both full scale and performance (nonverbal) IQ scores on the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III). One might expect that this involvement of the hippocampus in FXTAS would contribute to greater impairment of primary memory, but as noted previously, it is only in the later stages of the disorder that amnestic deficits show a more typically hippocampal pattern—initially, it seems that problems with memory are primarily secondary to the dysexecutive syndrome (Brega et al. 2008). Evidence of altered neural activity in carriers with FXTAS during a working memory task, as discussed in the previous section,

provides observable evidence of the neural manifestation of the dysexecutive syndrome (Hashimoto et al. 2011a). The relationship between grey matter loss in anterior cingulate cortex and left inferior frontal cortex and working memory deficit in premutation carriers provides further evidence of insult to executive function associated brain regions in FXTAS (Hashimoto et al. 2011b). Additionally, a preliminary study of white matter integrity in premutation carriers identifies a relationship between alterations in microstructure in genu and splenium with executive dysfunction and decreased processing speed (Filley et al. 2015).

In addition to cognitive deficits, the neuropsychological phenotype in FXTAS includes increased rates of mood and anxiety disorders (Besterman et al. 2014; Bourgeois et al. 2011). Volumetric analysis reports correlation between decreased hippocampal volume and increased psychological symptoms (including symptoms of anxiety and depression) in males with FXTAS (Adams et al. 2010). Hashimoto et al. (2011b) reported a significant relationship between depression and obsessive-compulsive symptoms with grey matter loss in the left amygdala in male premutation carriers (with and without FXTAS).

MRI Findings in Females with FXTAS

Because the incidence of FXTAS in females with the premutation is lower than in males, fewer studies have focused on these individuals. One such study examined.

Fifteen female premutation carriers affected by FXTAS as compared to 20 unaffected female carriers, 11 female controls with a normal FMR1 allele, 36 affected male carriers, 25 unaffected male carriers, and 39 male controls (Adams et al. 2007). Female and male carriers with FXTAS were matched on duration of disease. Reduced brain volumes and increased white matter disease were associated with the presence of FXTAS in females compared with female controls. Females affected by FXTAS also demonstrated reduction of cerebellar volume relative to controls although the magnitude of the reduction was less pronounced than in the male FXTAS patients. The MCP sign was also observed in females affected by FXTAS, but the incidence was lower (13 %) than that seen in the affected males (58 %). Renaud et al. (2014) did not observe the MCP sign in their small sample of females affected by FXTAS; however, hyperintensities in the splenium of the corpus callosum were observed in 3 of 5 females, an incidence comparable to that observed in males. Also, while significant associations were observed between reduced cerebellar volume and both increased severity of FXTAS symptoms and increased length of the CGG repeat expansion in male premutation carriers, this association was not observed for the females with FXTAS, although the analysis did not correct for activation ratio in females (Adams et al. 2007). Overall, these findings indicate that while radiologic findings (i.e., brain atrophy and white matter disease) observed in females affected by FXTAS are similar to those observed in men with the disease, the abnormalities are less pronounced than those seen in males with FXTAS.

Summary

Much is known about the radiological signs and the pattern of cerebral and white matter atrophy in FXTAS. A great deal of knowledge has also been gained about the correlates of these brain abnormalities with both behavioral and molecular variables. Future research directions in this domain include searching for early structural and functional brain biomarkers for FXTAS that will allow us to identify presymptomatic carriers as well as understand what risk factors (vascular, behavioral, or otherwise) may predispose premutation carriers to developing the disease. Longitudinal and multisite consortium studies will allow for the identification of potential biomarkers and risk factors, as well as better characterize the progression of FXTAS in premutation carriers.

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Chapter 5
The Pathology of FXTAS

Veronica Martinez Cerdeno and Claudia Greco

Abstract In 2002, a syndrome of tremor, ataxia, cognitive decline, and the presence of unique ubiquitin staining intranuclear inclusions in the brain was discovered in premutation males carrying an expansion of between 55 and 200 CGG trinucleotide repeats on the *FMR1* gene. This clinical syndrome is now known as fragile X-associated tremor/ataxia (FXTAS) and has been found in both male and female carriers of the expanded premutation allele. The goal of this chapter is to summarize what is known about the anatomical pathology associated with the fragile X premutation and particularly in those individuals with FXTAS. Neuropathology in FXTAS was initially found in the central nervous system, but recent evidence has demonstrated pathological features, including intranuclear inclusions, in the peripheral nervous system, the enteric nervous system, and the neuroendocrine system. The precise cellular dysfunctions that underlie these pathologic features are currently under intense investigation with the goal of prevention and treatment of this devastating disorder.

Keywords Intranuclear inclusions • RNA toxicity • White matter disease • MPC signs • Parkinson's disease

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Abbreviations

DRPLA	Dentatorubropallidoluysian atrophy
SBMA	X-linked spinobulbar muscular atrophy (Kennedy's disease)
NIID	Neuronal intranuclear inclusion disease
SCA	Spinocerebellar ataxia
AD	Alzheimer's disease
PD	Parkinson's disease
5′ UTR	5′ untranslated region
MS	Multiple sclerosis
FXTAS	Fragile X-associated tremor/ataxia syndrome
FMR1	Fragile X mental retardation gene
FMRP	Fragile X mental retardation protein
H&E	Hematoxylin and eosin stain
HD	Huntington's disease
PAS	Periodic acid-Schiff stain
LFB	Luxol fast blue stain
MCP	Middle cerebellar peduncle
IF	Intermediate filament
NF	Neurofilament

Introduction

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset, progressive neurodegenerative disorder that affects many carriers of an *FMR1* premutation, an expanded trinucleotide repeat sequence (CGG) in the 5′ untranslated region (5′ UTR) of the *FMR1* gene. This gene is polymorphic, and in unaffected individuals there are roughly 5–45 CGG repeats, while individuals with a premutation carry an allele of 55–200 CGG repeats and show a two-to-eightfold increase in levels of the *FMR1* mRNA (see Chap. 6).

Patients with FXTAS typically show cerebellar ataxia, tremor, cognitive deficits, peripheral neuropathy, autonomic dysfunction, and psychiatric involvement (see Chaps. 1 and 3). The disorder is thought to arise from a toxic RNA gain of function that is caused by overexpression of the expanded CGG *FMR1* mRNA in premutation carriers (Allen et al. 2005; Kenneson et al. 2001; Tassone et al. 2000). Magnetic resonance imaging (MRI) also shows that patients with FXTAS have mild to moderate brain atrophy in both cerebrum and cerebellum and white matter changes in cerebrum and cerebellum. Increased T2 signal intensity in the middle cerebellar peduncles (MCP) is commonly found in subjects affected by FXTAS (see Chap. 4) (Brunberg et al. 2002). Finally, the neuropathological hallmark of FXTAS is the presence of eosinophilic intranuclear inclusions in both neurons and astrocytes. These inclusions are found throughout the brain and in the autonomic nervous

system as well as in non-nervous system tissues (e.g., pancreas). In light of these unique findings, the proposed diagnostic criteria for FXTAS include the presence of intranuclear inclusions as a major criterion (Hagerman and Hagerman 2004; Jacquemont et al. 2004).

Intranuclear Inclusions

Intranuclear inclusions are the distinctive pathological finding among premutation carriers affected by FXTAS. They have also been observed in a knock-in mouse models of the *FMR1* premutation (see Chap. 8). Observed initially in human brain tissues (Greco et al. 2002), eosinophilic intranuclear inclusions are widely distributed in both neurons and astrocytes, being found in many different regions throughout the brain, including the frontal cortex, hippocampus, ependymal cells, choroid plexus, brainstem nuclei, and cerebellum (Greco et al. 2002, 2006). Given the significance of clinical symptomology related to the limbic system, it is important to note that the highest percentage of inclusions in FXTAS cases is in the hippocampus. Immunohistochemically, these inclusions stain positive for ubiquitin, lamin A/C, and a number of heat-shock proteins (Iwahashi et al. 2006). They stain negative for tau isoforms, α-synuclein, and polyglutamine peptides and appear to reflect a new class of nuclear inclusion disorder as compared to other triplet repeat disorders, such as Huntington's disease and some of the spinocerebellar atrophies (SCA). FXTAS is also distinct from neuronal intranuclear inclusion disorder (NIID) (Hagerman and Hagerman 2004). Furthermore, these inclusions do not contain any single predominant protein species; the most prominent protein accounts for only roughly 7 % of the total protein mass (Iwahashi et al. 2006). Also noteworthy is that in patients with FXTAS, and in contrast to patients with CAG repeat degenerative disorders and inclusions, the protein product of the *FMR1* gene, FMRP, is structurally normal and present at relatively normal or only slightly reduced expression levels, due to the fact that the expanded CGG repeat occurs in a non-coding portion (5′ UTR) of the gene.

A highly efficient, flow-based isolation and purification of inclusions from postmortem FXTAS brain tissues has allowed for mass spectrometric analysis of the entire protein complement of the isolated inclusions as well as follow-up immunohistochemical analysis to conclusively identify more than 20 inclusion-associated proteins. Several proteins appear to be ubiquinated and/or polyubiquinated in these purified inclusions, but ubiquinated proteins are the minority (Iwahashi et al. 2006). Ubiquitin is present within intracellular aggregates of a wide range of neurological disorders, not just FXTAS (Woulfe 2008). In the case of FXTAS, ubiquitin, a proteosomal degradation product, is utilized as a marker for isolation or detection of intranuclear inclusions by immunostaining (Greco et al. 2002, 2006; Iwahashi et al. 2006). Among the proteins identified within the inclusions are the RNA-binding protein, hnRNP A2, several intermediate filament (IF) proteins, including lamin A/C, the small heat-shock protein αB-crystallin, α-internexin, and other neurofilament

(NF) proteins. The *FMR1* mRNA is present, but only as a minor component within the inclusions (Tassone et al. 2004b), and FMRP has not yet been found to be present in the inclusions. All of the proteins found in the inclusions are potential candidates for involvement in the RNA gain of function that may underlie FXTAS pathology (Hagerman and Hagerman 2004; Iwahashi et al. 2006).

The time course of inclusion formation relative to clinical onset of disease is not yet known, nor is it understood whether the intranuclear inclusions are directly causative of FXTAS pathophysiology and symptomology, or simply a reflection of the progression of the disorder. If the inclusion materials are active or neurotoxic, they may contribute directly to damage to the nervous system. However, it is also possible that the intranuclear inclusions may represent a protective mechanism, serving as a repository for disabled enzymes and their products as has been proposed for Huntington's disease (Bowman et al. 2005). Intranuclear inclusions have also been reported in a fragile X premutation female carrier that showed no clinical symptoms associated with FXTAS (Tassone et al. 2012). These data suggest that the presence of intranuclear inclusions may be a consequence of, but not sufficient to lead to an FXTAS diagnosis (Tassone et al. 2012). Inclusions have been also described in a fragile X mosaic male (Pretto et al. 2013). A very low number of inclusions have been also detected in three older adults with FXS. These low levels suggest that the clinical course in these three subjects would not have been influenced by contributions from RNA toxicity (Hunsaker et al. 2011). The youngest deceased case of FXTAS (stage 2) described to date is a 36-year-old male that presented with both neuronal and astrocytic inclusions. The number of cells with inclusions and its size was comparable to that in older cases. These data indicate that even FXTAS is thought to be a disorder of aging in carriers of *FMR1* premutation, it can occur earlier in adult life, particularly if another disease process is occurring, such as substance abuse (as in this case) that may exacerbate the pathological process of FXTAS (Martinez-Cerdeno et al. 2015).

Recently, the presence of inclusions positive for a polyglycine containing FMRP protein (FMRpolyG) have been described in the CNS and non-CNS organs in FXTAS. Todd and colleagues (Todd et al. 2013) demonstrated that through initiation at a near-ATG codon located in the 5′UTR of the FMR1 gene a polyglycine-containing protein, FMRpolyG, is expressed. This protein accumulates in brain of FXTAS patients. It is also present in ubiquitin-positive inclusions in Drosophila, cell culture, and mouse disease models. Using two novel antibodies, they confirmed the existence of FMRpolyG-positive intranuclear inclusions in postmortem non-CNS material of a premutation carrier with FXTAS. Furthermore, the described colocalization of FMRpolyG and ubiquitin is found in the vast majority of inclusions. The presence of FMRpolyG-positive intranuclear inclusions in heart, kidney, adrenal gland, and thyroid is consistent with the unexplained medical comorbidities reported in some patients with FXTAS, including thyroid disease, cardiac arrhythmias, hypertension, migraine, impotence, and neuropathy (Buijsen et al. 2015). FMRpolyG have been also described in ovarian stromal cells of a woman with FXPOI but not in the ovaries of control subjects. The FMRpolyG-positive inclusions colocalized with ubiquitin-positive inclusions. Similar inclusions were

also observed in the pituitary of a man with FXTAS but not in control subjects. Similarly, ovaries of exCGG-KI mice, but not wild-type mice, contained numerous inclusions in the stromal cells that stained for both FMRpolyG and ubiquitin (Buijsen et al. 2015).

It is unclear whether the *FMR1* premutation predisposes individuals to or accelerates the course of other degenerative diseases of the central nervous system (or vice versa), being this a topic of active investigation. A number of FXTAS cases that have come to autopsy showed Lewy body formation in the substantia nigra, whether or not Parkinson's disease (PD) was clinically identified (Greco et al. 2002). An additional report described an elderly woman with cognitive impairment that was attributed to early AD that had both manifestations of FXTAS and premature ovarian failure (Al-Hinti et al. 2007). In addition, two females carrying the FXTAS premutation who suffered early onset Alzheimer's disease (AD) symptomology along with tremor and ataxia showed histopathological features of both AD and FXTAS (Greco et al., unpublished data). The superior and middle temporal gyri in these two women showed as high a percentage of intranuclear inclusions as seen in the hippocampus (unpublished data). In a reported case of concurrent FXTAS and multiple sclerosis (MS), the patient showed patchy and diffuse signal intensity alterations in white matter on T2-weighted MRI scans. Histologically, there were numer-

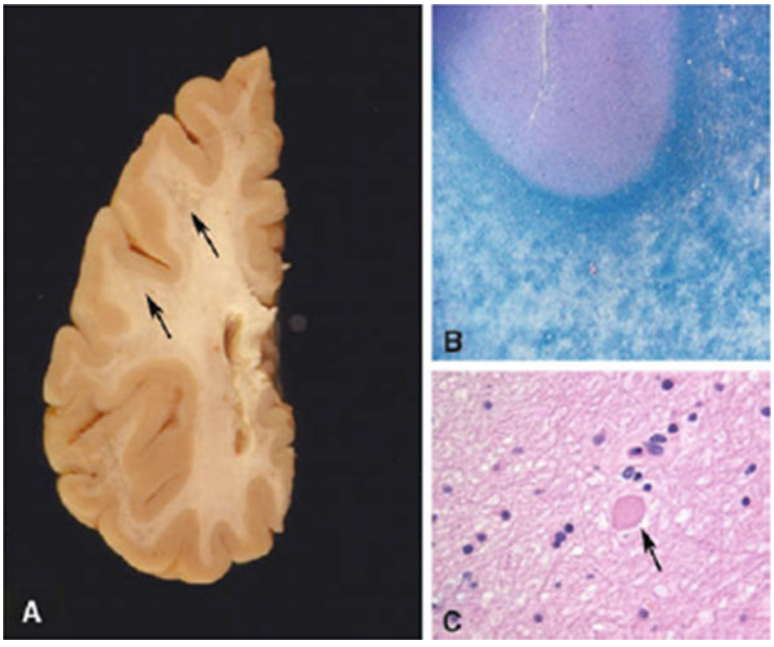

Fig. 5.1 (**a**) Severe subcortical white matter degeneration, as seen in some cases of FXTAS autopsy brains; (**b**) Corresponding patchy white matter loss affecting cortical U-fibers (×40, LFB–PAS stain); (**c**) Occasional swollen axons can be identified in cerebral and cerebellar white matter and middle cerebellar peduncles

ous regions of demyelination as well as the presence of intranuclear inclusions (Fig. 5.1). Recently, the first case of case of *FMR1* premutation with Prader-Willi Phenotype (PWP) and FXTAS has been described. This patient presented with eosinophilic and ubiquitin-positive intranuclear inclusions throughout his brain, as is common in FXTAS and suffered multiple architectural cortical abnormalities that may be related to neuroblast migration problems during cerebral cortex prenatal development, including abnormal gyration, mild ventricle enlargement, thin cortex, thin corpus callosum, and a left small inferior olive (Martínez-Cerdeño et al., unpublished data).

Brain Pathology

Gross Brain Pathology

In FXTAS, white matter disease is broad within the cerebrum and cerebellum, and is accompanied by a mild to moderate cortical atrophy and ventriculomegaly. The brain also presents with brainstem atrophy, especially of the pons. There are no notable gross structural changes in the spinal cord. When Parkinson's disease is concurrent, the substantia nigra is pale. When Alzheimer's disease is concurrent, cortical atrophy is often more prominent than that usually seen in FXTAS alone (Greco et al. 2002). In a reported case of concurrent FXTAS and multiple sclerosis, the patient showed patchy and diffuse signal intensity alterations in white matter on T2-weighted MRI scans. Histologically, there were numerous regions of demyelination as well as the presence of intranuclear inclusions (Fig. 5.1), (Greco et al. 2008). In a high percentage of FXTAS cases, increased T2 signal intensity is present in the middle cerebellar peduncles and can also be seen in the deep cerebellar white matter and brainstem (Brunberg et al. 2002), (See Chap. 4).

Microscopic Brain Pathology

Inclusions on H&E stains are discrete, hyaline-appearing, eosinophilic, round to slightly ovoid bodies (Fig. 5.2). They typically measure 2–5 μm in diameter and are single within a nucleus, except for those in Purkinje cells that are often twin inclusions (Ariza et al., 2016). They are periodic acid-Schiff (PAS), silver, tau, and neurofilament (NF) negative but stain positively for ubiquitin. Although inclusions have been identified in neurons throughout the brain, they have not been seen in Betz cells. Purkinje cell loss beyond that expected with otherwise normal aging and axonal swellings/torpedoes are commonly seen in FXTAS. No correlation have been found between the loss of Purkinje cells and the presence of single or twin inclusions within Purkinje cells (Ariza et al., 2016). Bergmann gliosis accompanies Purkinje cell loss in the cerebellum (Greco et al. 2002, 2006). Inclusions are also

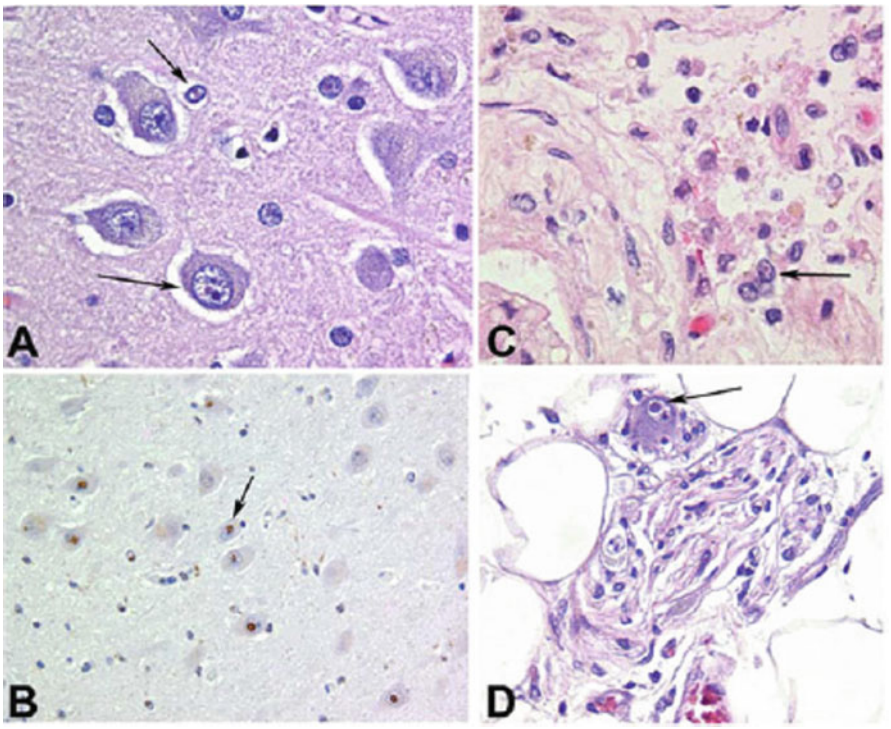

Fig. 5.2 (**a**) Neuronal and astrocytic intranuclear inclusions, CA4 (×400, H&E stain). (**b**) Ubiquitin immunoperoxidase stain showing numerous intranuclear inclusions, CA4, of hippocampus (×200). (**c**) Intranuclear inclusions, similar to those seen in the nervous system, are identified in Leydig cells (testosterone producing) of the testicles (×200, H&E). (**d**) Epicardial fat pad autonomic ganglion cell harboring an intranuclear inclusion (×200, H&E)

present in neurons of the dentate nucleus, and neurons and astrocytes throughout the cerebellum. When clinical PD has been diagnosed, cytoplasmic Lewy bodies are seen in pigmented neurons of the substantia nigra, and when FXTAS coexists the intranuclear inclusions can be seen in the pigmented neurons of the substantia nigra, whether or not cytoplasmic Lewy bodies are present. Two cases of PD and the characteristic radiological (MCP) sign demonstrated widespread p62-positive and ubiquitin-positive and 1C2-negative neuronal and glial intranuclear inclusions with mild Purkinje cell depletion consistent with FXTAS. There was also loss of pigmented neurons in the substantia nigra with α-synuclein-positive Lewy bodies (De Pablo-Fernandez et al. 2015). In both symptomatic male and female premutation carriers who also carried a diagnosis of AD, the intranuclear inclusions of FXTAS could be seen in pyramidal neurons of the hippocampus that also contained neurofibrillary tangles. Tree women with FXTAS plus dementia showed the presence of intranuclear inclusions and sufficient number of cortical amyloid plaques and neurofibrillary tangles to make AD a highly likely cause of dementia. A fourth case had dementia with cortical Lewy bodies and inclusions (Tassone et al. 2012).

In astrocytes, intranuclear inclusions are usually surrounded by a clear halo, although this may be an artifact of tissue preparation. They are present diffusely in both protoplasmic astrocytes of gray matter and fibrillary astrocytes of white matter of the brain and spinal cord. They are also seen in pituicytes, the modified astrocytes of the posterior pituitary gland (Louis et al. 2006). Inclusions are also present in cells of the choroid plexus and ependyma, both of which have astrocyte lineages. In contrast, they are rarely present in microglia and have not been identified in oligodendrocyte nuclei or endothelial cells of the brain. The appearance of intranuclear inclusions is similar between males and females and between brain and spinal cord. While the spinal cord is otherwise grossly unremarkable, intranuclear inclusions have been identified in astrocytes and autonomic neurons of the intermediolateral column of the spinal cord but not in anterior horn cells (Gokden et al. 2008; Greco et al. 2002, 2006). Gokden et al. (2008) have also observed intranuclear inclusions in paraspinal sympathetic ganglia.

The appearance of intranuclear inclusions by electron microscopy is similar in neurons and astrocytes and appears as non-membrane-bound collections of granulofilamentous material (Greco et al. 2002). The filaments appear as straight rod-like proteins arranged in a haphazard manner (Gokden et al. 2008; Greco et al. 2002). The ultrastructural appearance of these inclusions is, however, otherwise not particularly informative.

Morphometric Analysis: Neuronal and Inclusion Counts

Percentages of neurons and astrocytes containing intranuclear inclusions were originally determined only in one study, for frontal cortex, hippocampus, and the ventral pontine region of 8 male FXTAS patients and 10 normal (no neurological disease), age-matched control subjects. Quantification of inclusions was carried out using a computer-based imaging and cell-counting system (StereoInvestigator, MBI, Inc.,

Table 5.1 Percent of neurons and astrocytes with intranuclear inclusions in FXTAS patients

Brain region	Percentage of neurons with inclusions	Percentage of astrocytes with inclusions
Frontal cortex		
Gray matter	4.4 ± 1.4	16.7 ± 3.8
White matter	22.0 ± 6.4	5.0 ± 1.9
Hippocampus		
Pyramidal neurons	10.1 ± 2.8	10.3 ± 4.2
Granule cells	2.1 ± 1.0	26.6 ± 6.1
Hilar neurons	11.0 ± 3.6	28.3 ± 6.2
Pontine nuclei	0.2 ± 0.1	20.8 ± 3.1

Summary of data contained in Tables 3–5 in Greco et al. (2006)

Williston, VT) on H&E-stained slides. The number of neurons and astrocytes with intranuclear inclusions (actual counts and percentages) is presented in Table 5.1 (summary of Tables 3–5 from (Greco et al. 2006)). No inclusions were seen in control cases. Inclusions were later described in Pukinje neurons in 65% of the FXTAS cases analyzed (26 cases). Of those, more than half of the cases (9/17) possessed twin adjacent nuclear inclusions. For the 17 cases with inclusions, Purkinje cells containing inclusions accounted for 9.1± 0.9 % of the total Purkinje cells. Those Purkinje cells with twin inclusions comprised 16.7 ± 2.7 of all Purkinje cells with inclusions (Ariza et al, 2016). The presence of inclusions in Purkinje cells was correlated with age). Other quantification studies include that of the youngest deceased case ever diagnosed with FXTAS, a man in his 30s, that contained 9.5 % of cells in the prefrontal cortex (14.2 % astrocytes; 4.8 % neurons), 6.4 % in CA1 (18 % astrocytes; 9.6 % neurons), 5.7 % in caudatum (20 % astrocytes; 6.4 % neurons), and 5.3 % in cerebellum (3.2 % astrocytes; 6.0 % neurons) (Martinez-Cerdeno et al. 2015). The case with FXTAS and PWP presented inclusions within the cerebral and cerebellar cortices in 14 % of cells, while the hippocampus had 3 % in the molecular layer, 14 % in the pyramidal layer, and 11 % in the granular layer. Intranuclear inclusions in astrocytes were massive, occupying up to 80 % of the nucleus, while in neurons the inclusions were smaller and about the size of the nucleolus (Martínez-Cerdeño et al., unpublished data).

In general, more and bigger intranuclear inclusions were observed in astrocytes than in neurons (the hippocampal CA1 subregion was an exception, but the pyramidal cell layer was counted, which contains relatively few astrocytes compared to neurons relative to the cortex), although there was a great deal of variability across subjects. Statistical correlations (Spearman's rho) were calculated between histological findings and molecular measures. Significant positive correlations were present between the percentages of both neurons and astrocytes with inclusions in several brain regions and the number of CGG repeats. However, correlations between percentage inclusions and peripheral blood leukocyte *FMR1* mRNA or FMRP levels were not statistically significant. This last observation is not surprising in view of the large differences between expression levels in brain and blood and the region-specific differences in *FMR1* mRNA levels in brain (Tassone et al. 2004a). Most striking was the clinical–molecular correlation that showed a significant decrease in age of death with increasing CGG repeat length (i.e., the greater the CGG repeat number, the earlier the age of death; (Greco et al. 2006)).

White Matter Pathology

White matter changes seen on MRI studies include nonspecific, subcortical, patchy regions of increased T2 signal intensity in the cerebrum. In a high percentage of FXTAS cases, increased T2 signal intensity is present in the middle cerebellar peduncles (see Chap. 4) (Brunberg et al. 2002) and can also be seen in the deep cerebellar white matter and brainstem.

When these regions are examined microscopically using histologic and immunochemical stains, abnormal areas of white matter show spongiosis, axonal degeneration, and myelin loss. The same histological features are identified in damaged white matter of the cerebellum (Greco et al. 2002, 2006). In cases with the most severe cerebral white matter changes, scattered fibrillary astrocytes are greatly enlarged by irregular expansion of cytoplasm that contains lysosomal debris. These same cells may also contain intranuclear inclusions. Rare axonal spheroids have been identified in spongiotic middle cerebellar peduncles on H&E and neurofilament stains. The middle cerebellar peduncles may also show myelin pallor on LFB–PAS stain (Greco et al. 2006).

Peripheral Nervous System

While the intranuclear inclusions of FXTAS were first identified in neurons and astrocytes of the brain in 2002 (Greco et al. 2002), systemic locations of the inclusions in the peripheral nervous system and other tissues are rapidly being cataloged and published in the medical literature.

Autonomic System

Inclusions have been observed in paraspinal sympathetic ganglion, ganglion cells of adrenal medulla, ganglion cells of the myenteric plexus of the stomach, and ganglion cells of a subepicardial ganglion. Also, intranuclear inclusions have been identified in dorsal root ganglion neurons in the spinal cord (autonomic neurons), but not in the ventral root (Gokden et al. 2008). Symptoms corresponding to this autonomic pathology may include mega-esophagus, constipation, bladder spasms, orthostasis, hypertension, and sexual dysfunction (see Chaps. 1 and 9).

Peripheral Nerve

Nonspecific features of axonal degeneration have been seen in nerve examined at autopsy. Inclusions have not been observed by light microscopy. Clinically, neuropathic features are seen in male premutation carriers (Berry-Kravis et al. 2007), and peripheral neuropathy of variable severity is noted in individuals with FXTAS with reduced peripheral nerve conduction velocity (Soontarapornchai et al. 2008). The possible causes of this dysfunction are unknown.

Skeletal Muscle

Light microscopy, including histochemical and enzyme staining, has shown no pathological changes. Ultrastructural examination has yielded no distinctive abnormalities.

Neuroendocrine

In a limited number of cases (one male and one female), intranuclear inclusions within the anterior and posterior pituitary have been identified and may be associated with dysregulation of neuroendocrine function (Gokden et al. 2008; Greco et al. 2007; Louis et al. 2006). Similar findings have been made for the CGG KI mouse (see Chap. 8). This observation is of particular interest in view of the elevated cortisol levels found in FXTAS, as well as an increased incidence of anxiety disorders and depression (Bourgeois et al. 2009; Hunter et al. 2008; Rodriguez-Revenga et al. 2008).

Testicular pathology has been documented in two cases of FXTAS stained with H&E, including tubular fibrosis, decreased numbers of Leydig cells, and decreased spermatogenesis. Sertoli cells were abundant in the tubules along with a scant number of germ cells and spermatozoa that were remnants of germ maturation, but these changes were comparable to those seen in normal age-matched controls. There were also eosinophilic intranuclear inclusions in a small percentage of the Leydig cells in both cases as well as in the myoid cells of the tubular walls. Inclusions within the Leydig cells may be related to decreased levels of testosterone in some younger males with FXTAS who suffer premature erectile dysfunction. The presence of intranuclear inclusions in myoid cells in testicular connective tissue compartments in FXTAS is intriguing. The tunica propria of the testicle is a component of the tunica albuginea, and it is the middle of the three layers of the fibrous capsule beneath the scrotal skin that protects and supports the testes. Among other cellular components, the tunica albuginea contains myofibroblasts. In the tunica propria smooth muscle cells are involved in contractile and transport functions. Inclusions in these cells suggest that other cell populations outside of the nervous system may also have inclusions. This finding raises the possibility of identifying easily accessible diagnostic tissue for biopsy, and such tissue samples could be used for diagnostic purposes or for monitoring therapeutic responses to treatment (Greco et al. 2007). No data is available about ovary pathology in FXTAS; however, premutation carrier mice show a faster loss of follicles, many oocytes with aberrant nuclear accumulation of FMRP and elevated levels of ubiquitination. Furthermore, follicles are smaller and have fewer granulosa cells (GCs) than normal (Hoffman et al. 2012). Studies in human should follow up to better understand the pathology of the ovary in FXTAS. For more details, see Chap. 10 and 11.

Other Related Pathology

Other pathology of the FXTAS brain include prominent iron accumulation in the choroid plexus and putamen, and a milder iron accumulation in cerebellum, together with an alteration of the expression levels of proteins related to iron metabolism (Ariza et al. 2015; Rogers et al. 2016). Iron depositions are present in postmortem choroid plexus in FXTAS. In addition, transferrin levels are decreased in the epithelial cells, and transferrin receptor 1 distribution is shifted from the basolateral membrane (control) to a predominantly intracellular location (FXTAS). In addition, ferroportin and ceruloplasmin are markedly decreased within the choroid epithelial cells. These data indicate an alteration in iron transport and metabolism in the brain in FXTAS. In addition, these alterations have implications not only for understanding the pathophysiology of FXTAS, but also for the development of new clinical treatments that may incorporate selective iron chelation (Ariza et al. 2015).

FXTAS brain also presents with a reduction of proteins related to glutamate signaling (Pretto et al. 2014). The expression levels of the metabotropic glutamate (Glu) receptor 5 and the Glu transporter excitatory amino acid transporter 1 are reduced in the postmortem cerebellum of FXTAS, with higher CGG repeat number having greater reductions in both proteins. These data suggest a dysregulation of Glu signaling in premutation carriers, which could likely contribute to the development and severity of FXTAS (Pretto et al. 2014).

Summary

Since the initial discovery in 2002 that male premutation carriers with a clinical syndrome of tremor, ataxia, and cognitive decline showed a unique intranuclear inclusion disorder in pathological studies, there have been further studies elucidating the histologic, molecular, and biochemical features of the inclusions. The peripheral nervous system, specifically the autonomic system, is clearly involved, as is the neuroendocrine system. Cellular dysfunctions that underlie these pathologic features are currently under intense investigation with the goal of prevention and treatment of this devastating disorder.

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Chapter 6
The Molecular Biology of Premutation Expanded Alleles

Flora Tassone, Chantal Sellier, Nicolas Charlet-Berguerand, and Peter K. Todd

Abstract Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late adult onset neurodegenerative disorder that mainly affects male carriers of an allele of 55–200 CGG repeats in the *FMR1* gene (premutation). FXTAS symptoms include progressive intention tremor, gait ataxia, neuropathy, psychiatric symptoms, cognitive decline, and autonomic dysfunctions. Neuropathological features of FXTAS include global cerebral and cerebellar atrophy, spongiform changes of white matter, marked Purkinje cell dropout and presence of ubiquitin-positive intranuclear inclusions throughout the brain. In contrast to fragile X Syndrome (FXS), FXTAS is associated with elevated expression of repeat containing *FMR1* mRNA, which binds to and sequesters specific RNA binding proteins and impedes their normal functions. In addition, the CGG repeat expansion triggers a non-canonical translational initiation event to produce a polyglycine containing protein (FMRpolyG) that accumulates in patients brains. Here we discuss these and other putative molecular mechanisms for FXTAS pathogenesis with a focus on recent findings.

Keywords RAN translation • RNA disease • Transcription • Antisense • Neurodegenerative disease • Disease mechanisms

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Introduction

Although the developmental disorder, fragile X syndrome (FXS), is almost always caused by CGG repeat expansions exceeding 200 CGG repeats (full mutation range), with hypermethylation of the promoter region and consequent transcriptional silencing (Fu et al. 1991; Oberlé et al. 1991; Pieretti et al. 1991; Verkerk et al. 1991; Yu et al. 1991), the adult onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS), almost exclusively affects carriers of premutation alleles (55–200 CGG repeats) with nearly all cases having alleles that exceed ~65–70 CGG repeats (Jacquemont et al. 2006). Premutation alleles are generally unmethylated but several studies have reported on the presence of premutation alleles partial methylated even in the lower premutation size range and in some case the methylation status correlated with the severity of the observed clinical involvement (Allingham-Hawkins et al. 1996; Pretto et al. 2014c; Tassone et al. 1999). Furthermore, several studies have reported FXTAS in individuals with a partially methylated full mutation (Loesch et al. 2012; Pretto et al. 2014a; Santa Maria et al. 2013) and also in individuals with smaller alleles in the "gray zone" or intermediate range (45–54 CGG repeats; normal <45 CGG repeats) (Hall et al. 2012; Liu et al. 2013).

Concerning instability of the CGG repeat, alleles in the normal, gray, and premutation size ranges generally have one or more AGG trinucleotides within the CGG repeat tract, typically spaced with intervals of 8–11 intervening CGG repeats (e.g., 9-10-10 pattern). However, these intervening repeats are generally lost with larger CGG repeat sizes within the premutation size range (Kunst and Warren 1994; Zhong et al. 1996), which may result in the loss of repeat length stability (Zhong et al. 1995). Interestingly, expansion from a premutation allele to a full mutation allele occurs only during maternal transmission; that is, transmission of a full mutation allele never occurs from the father, even if the father is a carrier of a full mutation allele (Nolin et al. 2003). Although the presence of AGG interruptions within the CGG repeat does not affect either the expression of the *FRM1* mRNA (Yrigollen et al. 2012, 2014) or its translation (Ludwig et al. 2009), it has been demonstrated to play a key role in the stability of the repeat (Nolin et al. 2013; Yrigollen et al. 2012, 2014). Importantly, Yrigollen et al. demonstrated that the risk of expansion from premutation to full mutation alleles during maternal transmission decreases by increasing number of AGG interruptions. In addition, the risk of instability of an intermediate or premutation allele during maternal or paternal transmission is reduced with the presence of AGG interruptions, as is the magnitude of size change that occurs during transmission (Nolin et al. 2013; Yrigollen et al. 2014). Using logistic regression of 710 maternal transmissions and considering the total length of the CGG repeat allele, the number of AGG interruptions, and the age of the mother at childbirth, a model for measuring the risk of expansion to a full mutation during maternal transmission was calculated. This model was determined to be more suitable than that which considered pure CGG stretch instead of total length, confirming the previous data on a smaller sample (Yrigollen et al. 2012).

The prevalence of full mutation is approximately 1 in 5000 males and 1 in 2500–8000 females (reviewed in (Tassone et al. 2012a)). However, based on a recent systematic review and meta-analysis, approximately 1/7000 males and 1/11,000 females carry the full mutation (Hunter et al. 2014).

Reported frequencies of the premutation allele vary among studies of various populations. Canadian studies reported a premutation frequency of approximately 1 in 250 for females and 1 in 800 for males (Dombrowski et al. 2002; Rousseau et al. 1995). However, the frequency of the premutation in females in Israel is substantially higher, at approximately 1 in 113 (Toledano-Alhadef et al. 2001), and lower in an Asian population with a rate of 1 in 1674 premutation males in Taiwan (Tzeng et al. 2005). In a recent report, using the known frequency for premutation females (using the average of 1 in 126 from (Pesso et al. 2000; Toledano-Alhadef et al. 2001)) to calculate the expected number of full mutations, Hagerman (2008) determined that the expected frequency for premutation males would be 1 in 282 and for full mutation (both males and females) would be 1 in 2355. Interestingly, the former value is in agreement with what was observed in recent larger screening studies in which the prevalence of premutation alleles ranged between 150 and 210 in females and 290 and 430 in males (Hunter et al. 2014; Maenner et al. 2013; Tassone et al. 2012a) .

Clinical Involvement in *FMR1* Premutation Carriers

Over the last several years, there has been increasing awareness of the spectrum of the phenotypes associated with premutation alleles of the *FMR1* gene (Hagerman and Hagerman 2013). While the presence of a full mutation allele (>200 CGG repeats) generally result in FXS, with autism spectrum disorders in over 60 % of subjects (Harris et al. 2008), smaller repeat expansions in the premutation range give rise to several distinct forms of clinical involvement: (a) fragile X-associated tremor/ataxia syndrome (FXTAS) mainly in older males; (b) fragile X-associated primary ovarian insufficiency FXPOI in approximately 20 % of all carrier females; and may give rise to (c) behavioral, physical, emotional, and cognitive problems in some children who are premutation carriers, particularly at repeat sizes in the upper end of the premutation range.

FXTAS is mainly observed in males as demonstrated by the absence of significant differences between carrier females and controls when rated by CRST, ICARS, or UPDRS (Berry-Kravis et al. 2003; Jacquemont et al. 2004), or FXTAS Rating Scale (Leehey et al. 2007)—for details of use and findings with these rating scales in FXTAS the reader can see Chaps. 1 and 7. However, a percentage of female carriers clearly present clinical and neuropathological signs of FXTAS (Berry-Kravis et al. 2005; Jacquemont 2005; Peters et al. 2006; Zuhlke et al. 2004). In contrast to fragile X syndrome, both FXPOI and FXTAS appear to be generally confined to the premutation range with some exceptions (Liu et al. 2013; Loesch et al. 2012; Pretto et al. 2014c; Santa Maria et al. 2013). Moreover, while full mutations are

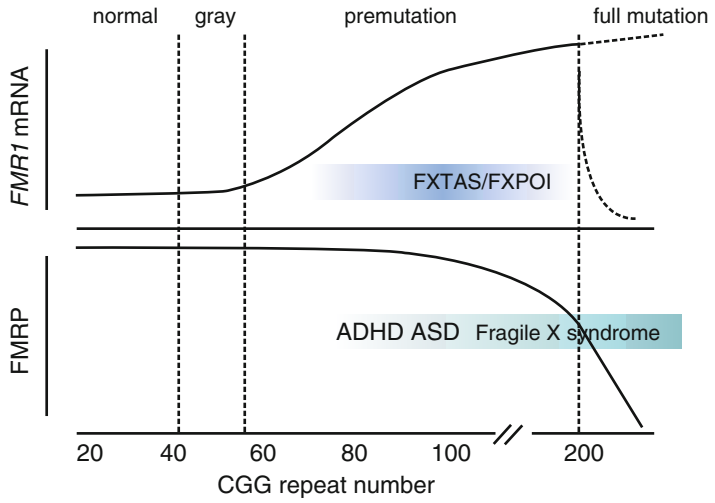

Fig. 6.1 FXTAS and FXPOI are largely confined to the premutation range and are thought to occur through an RNA toxic gain of function due to excess *FMR1* mRNA; however, patients with partially methylated full mutation alleles continue to express mRNA and are potentially at risk of developing FXTAS. By contrast, fragile X syndrome is caused by reduced/absent FMRP, due to silencing of the *FMR1* gene in the full mutation range. Features of the fragile X syndrome spectrum may also occur in the upper premutation range due to reduced protein production. *Dashed lines* for *FMR1* mRNA levels in the full mutation range reflect variations in degree of methylation; FMRP levels are reduced due to both *lower* mRNA levels and reduction in translation efficiency from the *upper* premutation range

characterized by the absence of *FMR1* mRNA, and consequently by the absence of FMRP, premutation carriers possess elevated levels of *FMR1* mRNA, with the extent of the increase depending upon the size of the premutation repeat expansion (Sellier et al. 2014; Tassone et al. 2000a). For the largest premutation alleles, FMRP levels are moderately reduced (up to twofold) due to a relative impediment to translation, demonstrated in both human and mouse (Ludwig et al. 2014; Primerano et al. 2002). A diagram of the relative levels of *FMR1* mRNA and proteins (FMRP) as a function of the number of CGG repeats and the association of clinical phenotypes is shown in Fig. 6.1.

The expanded CGG repeat, located in the 5′ UTR region of the *FMR1* gene, is now believed to cause the cellular toxicity in FXTAS, whereas FMRP is not thought to play a role in the neurodegenerative syndrome, since full mutation alleles (where FMRP is absent) are not associated with FXTAS. It is, however, possible that the decreased levels of FMRP could somehow act in concert with the effects of the toxic mRNA, particularly in the upper end of the premutation range, where lower FMRP levels are detected; however, many cases of FXTAS are observed for CGG repeats in the 80–100 range, where FMRP levels are not substantially reduced. Thus, it appears clear that FXS and FXTAS are caused by a CGG expansion in the *FMR1* gene but with completely different molecular mechanisms (RNA Gain of Function for FXTAS; Loss of FMRP function for FXS).

As noted above, FXTAS is more frequent in males, as would be expected for an X-linked disorder (Adams et al. 2007; Berry-Kravis et al. 2007; Coffey et al. 2008; Greco et al. 2008; Hagerman and Hagerman 2004b; Hagerman et al. 2004, 2007). In particular, female carriers are less likely to develop the disorder due to the protective effect of the expression of the normal allele in a portion of cells. Moreover, for those females who do experience features of FXTAS, expression of the normal *FMR1* allele is also likely to be responsible for the less severe clinical outcome (Hagerman and Hagerman 2004b; Zuhlke et al. 2004). X-inactivation effects are therefore predicted to have a protective effect toward the neurological features of FXTAS. Several reports, in which a correlation between the severity of clinical signs and the X-inactivation ratio has been observed, seem to be supportive of such theory (Berry-Kravis et al. 2005; Coffey et al. 2008; Jacquemont 2005). However, females can present with significant higher rates of muscle pain, thyroid problems, hypertension, and fibromyalgia (Coffey et al. 2008; Hagerman and Hagerman 2013; Hundscheid et al. 2003; Rodriguez-Revenga et al. 2009).

Although details of the medical, cognitive, psychiatric, and neurological phenotypes of FXTAS and in premutation carriers in general, are discussed more extensively in other chapters of this book, a few additional comments regarding phenotype are pertinent here. Borderline to mild cognitive deficits generally are observed when FMRP levels are moderately reduced, as is often the case for individuals with alleles in the high premutation range (>150 CGG repeats) or for individuals with alleles in the low full mutation range that remain unmethylated and therefore retain transcriptional and somewhat translational activity (Tassone et al. 2000b). Numerous phenotypic problems have been reported in premutation carriers that seem to begin in many cases in childhood. The most consistent deficits seen in such carriers of a large premutation include shyness, anxiety, social deficits, ADHD, and executive function deficits (Aziz et al. 2003; Chonchaiya et al. 2012; Cornish et al. 2005; Farzin et al. 2006). Thus, for the larger premutation alleles, cognitive involvement may reflect the combined effects of both RNA toxicity and lowered FMRP levels (Farzin et al. 2006; Goodlin-Jones et al. 2004; Tassone et al. 2000a). The occurrence of seizures in about 8–13 % of those with the premutation (Bailey et al. 2008; Chonchaiya et al. 2012; Hagerman and Stafstrom 2009), appears to be associated with the development of autism spectrum disorder (ASD) in males with the premutation (Chonchaiya et al. 2012). Further, immune mediate disorders, including hypothyroidism and fibromyalgia, sleep apnea, hypertension, and migraine are more common in premutation carriers than controls (Hagerman and Hagerman 2013), Finally, very recent studies of early development in infant premutation carriers has evidentiated the presence of processing deficits similar to infants with FXS. Specifically, a visual discrimination deficit in babies with the premutation that is similar to what seen in babies with the full mutation and that is significantly different from visual discrimination in age- and sex-matched control babies has been recently reported. These observation have suggested that spatiotemporal processing impairment may constitute an endophenotype in infant premutation carriers (Gallego et al. 2014). In addition, examination of cognitive, communication, and social–behavioral profiles has demonstrated subtle developmental differences in premutation infants as young as 12 months of age (Wheeler et al. 2015).

A number of studies have reported on the correlation between the length of the CGG repeat within the premutation range and the severity of clinical involvement in premutation carriers. These correlations are particularly strong with the neurological (FXTAS) phenotypes including the age of onset of tremor and ataxia (Tassone et al. 2007a), overall motor impairment (Leehey et al. 2007), severity of white matter disease and degree of brain atrophy (Cohen et al. 2006; Loesch et al. 2005), severity of neuropathic signs (Berry-Kravis et al. 2007), degree of neuropathy as measured by nerve conduction studies (Soontarapornchai et al. 2008), reduced cerebellar volume (Adams et al. 2007), and the percent of inclusions and age at death (Greco et al. 2006). These associations are discussed further in Chap. 5.

FMR1 Gene Structure

The *FMR1* gene consists of 17 exons spanning 38 kb of Xq27.3 (Eichler et al. 1993). The gene is expressed in many tissues; however, the highest expression of the 4.4 kb transcript is observed in brain, placenta, testis, lung, and kidney (Hinds et al. 1993). In both human, including fetal brain neurons, and mouse, extensive alternative splicing of the *FMR1* gene has been demonstrated by RT-PCR analysis, and several FMRP isoforms have been observed on Westerns (Ashley et al. 1993; Huang et al. 1996; Sittler et al. 1996; Verheij et al. 1993; Verkerk et al. 1993) due to alternative splicing in the carboxy-terminal half of the *FMR1* gene. Published studies have suggested that alternative splicing in the *FMR1* gene does not seem to be tissue specific, as similar ratios of transcripts were found in several fetal tissues, including brain and testis (Verkerk et al. 1993); however, quantitative approaches were not taken. The main splice variants observed in the *FMR1* gene involve the use of alternative splice acceptors in exons 12, 14, 15, and 17 (Ashley Ct et al. 1993; Verkerk et al. 1993). The existence of 16 different mRNA isoforms has been recently described in different tissues (Pretto et al. 2015). Although the relative abundance of these isoforms was reported to be significantly increased in premutations, interestingly the abundance of isoforms spliced at both exons 12 and 14, specifically *Iso10 and Iso10b,* containing the complete exon 15 and differing only in splicing in exon 17 was four to sixfold increased compared to controls. Based on their findings, the authors suggested that RNA toxicity is likely to arise from an increased level of all *FMR1* mRNA isoforms and such increase may have a functional relevance in the pathology of *FMR1*-associated disorders. Although the specific function of the splice variants is not known, four or five different FMRP isoforms have been described using FMRP-specific antibodies (Verheij et al. 1995) some of which act as shuttling proteins that transports their mRNA targets from the nucleus to the cytoplasm (Dury et al. 2013).

In addition to the presence of alternative splicing of the *FMR1* gene, a detailed analysis of the CG-rich, TATA-less, promoter region of the *FMR1* gene has revealed an influence of the CGG repeat with respect to initiation or the start of initiation. The existence of three distinct groups of transcriptional initiation sites, and the

distribution of these start sites, which is modulated by the number of CGG repeats in the downstream (5′ UTR) region, has been demonstrated in different tissues including lymphocytes and neuronal cells (Beilina et al. 2004; Tassone et al. 2011). While premutation alleles appear to preferentially express the longer *FMR1* mRNA (50 bp longer compared to the most frequent transcription initiation site in the normal alleles) (site II or site III), normal alleles appear to preferentially use the shorter transcripts initiating at site I (Beilina et al. 2004; Tassone et al. 2011). Interestingly, the nucleotide sequence of all three transcriptional initiation sites was found to be highly similar to the consensus sequence of pyrimidine-rich initiator (Inr) elements (consensus sequence YYAN(T/A)YY) (Javahery et al. 1994), which are usually located near the start site and have been implicated in transcription initiation in TATA-less genes (Chow et al. 1995). In addition, a fourth transcription initiation site, located between the previously identified sites I and II, was identified in brain tissues, suggesting the presence of a brain-specific transcription start site (Tassone et al. 2011). The dependency of alternative transcription initiation site usage on the CGG repeats length has also been observed in mice (Tassone et al. 2011).

Alternative polyadenylation site usage in the 3′UTR, which is implicated in the regulation of gene expression of many genes, has also been observed in the *FMR1* mRNA. Several different mRNA transcripts through the usage of different polyadenylation signals were identified in both human and mouse brain tissue (Tassone et al. 2011). Thus, the combination of both alternative 5′ and 3′ UTRs in addition the complexity of expression of the *FMR1* isoforms suggest their potential role in neuronal physiology, as well as in *FMR1*-associated disorders; however, it needs to be investigated.

Importantly, the differential usage of initiators in normal and premutation alleles may imply a possible variation in translational efficiency (post-transcriptional regulation) due to variation in 5′ structure and sequence. Indeed, the FMRP deficit observed, especially in the upper premutation range, is CGG dependent and is due to decreased translational efficiency. Reduced translational efficiency was observed both in cell lines and in transient transfection experiments using expanded alleles spanning the entire premutation range (Chen et al. 2003; Primerano et al. 2002). Similar findings are observed in two independently generated knock-in mouse models of FXTAS (Brouwer et al. 2008; Entezam et al. 2007; Iliff et al. 2013; Ludwig et al. 2014; Willemsen et al. 2003). To date, the precise mechanism by which the expanded CGG repeat impedes translation is not clear. Translation from the larger premutation alleles is expected to be severely inhibited by the predicted free energies of stabilization of the CGG repeat element. One interesting feature of the transition from normal to the premutation range is the gradual loss of AGG interruptions within the CGG element located in the 5′ UTR region of the *FMR1* mRNA.

As mentioned earlier, a few studies have shown that both transcription and translation expression levels (Ludwig et al. 2009; Tassone et al. 2007b; Yrigollen et al. 2012) of the *FMR1* mRNA are not influenced by AGG interruptions. Therefore, even if the presence of AGG repeats may modify the secondary/tertiary structure of the UTR region of the *FMR1* gene, such alterations do not seem to have an effect on transcription or represent a rate-limiting step in translational initiation.

Higher *FMR1* Transcription Rate in Premutation Alleles

While hypermethylation of the *FMR1* gene in full mutation alleles leads to transcriptional silencing, premutation alleles are generally unmethylated and, therefore, transcriptionally active. Moreover, *FMR1* message levels are two to tenfold higher than normal, particularly in the upper end of the premutation range (~100–200 CGG repeats), despite their reduced protein (FMRP) levels (Sellier et al. 2014). Both elevated mRNA levels (up to fivefold) and FMRP deficit were also observed in some fragile X males with a partially methylated full mutation, even for alleles greater than 300 CGG repeats (Tassone et al. 2000b).

Elevated *FMR1* mRNA levels observed in premutation carriers appear to result not from increased mRNA stability (Tassone et al. 2000a) but rather from increased transcriptional activity of the *FMR1* gene. This was best demonstrated by increased levels of run-on transcription in premutation alleles compared to normal (Tassone et al. 2007a). Nuclear retention of expanded repeat *FMR1* mRNA was not observed by RNA in situ hybridization experiments in patient derived lymphocytes (Tassone et al. 2007a) although this has not yet been evaluated in neurons. Elevated levels of *FMR1* messenger RNA and a deficit in translation efficiency in expanded alleles has been evaluated using in vitro translation experiments with a luciferase reporter mRNA containing the 5′UTR of the *FMR1* gene containing varying numbers of CGG repeats. Specifically, the translation efficiency gradually decreases with increasing CGG repeat number (Chen et al. 2003).

The augmented transcription of *FMR1* mRNA is associated with epigenetic alterations induced by the CGG repeat expansion itself (Todd et al. 2010). Specifically, ectopically expressed CGG repeat expansions are sufficient to elicit chromatin changes in a *Drosophila* model of the disease and similar changes are observed in patient derived cell lines. Interestingly, these alterations in local chromatin structure are dynamic and modifiable by genetic and pharmacologic means, suggesting that agents aimed at chromatin remodeling might have therapeutic potential in FXTAS (Todd et al. 2010). How exactly the repeat leads to these epigenetic alterations is unclear. Long tracts of CGG repeats are known to exclude nucleosomes in vitro (Wang et al. 1996). Should this also occur in vivo it could lead to enhanced transcription by enhancing access of transcription factors to the promoter. Alternatively, recent reports demonstrate that transcribed CGG repeats form R-loop structures that could also potentially trigger increased *FMR1* expression (Groh et al. 2014; Loomis et al. 2014; Usdin et al. 2014). Future work will be required to define the sequential pathway and proximal events that lead to transcriptional up-regulation.

ASFMR1: The Antisense Transcript at the *FMR1* Locus

Using RACE analysis and strand-specific RT-PCR, Ladd and collaborators (Ladd et al. 2007) identified an antisense transcript of the *FMR1* gene *(ASFMR1)* overlapping with the CGG repeat region. The *ASFMR1* is widely expressed in human

tissues, with a higher expression in brain and similarly to the *FMR1* gene, the expression appears to be influenced by the CGG repeat number as it is upregulated in peripheral blood leukocytes of premutation carriers and silenced in full mutation alleles. Moreover, *ASFMR1* transcript is alternatively spliced, polyadenylated, exported to the cytoplasm, and contains an open reading frame encompassing the CCG repeat that potentially encodes a polyproline peptide (Ladd et al. 2007). Shortly after this report, Khalil and collaborators described a second antisense transcript to *FMR1*, *FMR4*, which originates upstream of the *FMR1* start site and covers 2.4 kb of sequence (Khalil et al. 2008). Expression of *FMR4*, like that of *ASFMR1* and *FMR1*, is increased in brain from premutation individuals and silenced in individuals with the full mutation (Khalil et al. 2008). Importantly, *FMR4* overexpression increases cell proliferation while *FMR4* down-regulation induces apoptosis in vitro (Khalil et al. 2008). More recently, two novel transcripts arising from the *FMR1* locus, *FMR5* and *FMR6*, were identified (Pastori et al. 2013). *FMR5* is a sense-oriented long non-coding RNA (lncRNA) transcribed from approximately 1 kb upstream of the *FMR1* transcription start site. In contrast to *FMR1*, the *FMR5* transcript is not differentially expressed in human brain from unaffected individuals compared to full and premutation patients, suggesting that its transcription is independent of CGG repeat expansion (Pastori et al. 2013). *FMR6* is a spliced 600 nt long antisense transcript whose sequence is complementary to the 3′ region of *FMR1*. *FMR6* begins in the 3′UTR, ends in exon 15 of *FMR1*, and uses the same splice junctions as *FMR1*. Unexpectedly, the expression of *FMR6* is reduced in premutation carriers suggesting that abnormal transcription and/or chromatin remodeling occurs toward the distal end of the locus. However, the chromatin marks associated with the 3′ end of *FMR1* in premutation carriers are yet to be described and further studies are needed to determine the potential contribution of these long non-coding RNA to the variable clinical phenotypes associated with FXTAS and to the *FMR1*-associated disorders.

RNA Toxic Gain-of-Function Model

As stated above, FXTAS has been observed, with rare exceptions, only in premutation carriers (Loesch et al. 2012; Pretto et al. 2014c; Santa Maria et al. 2013). The absence of FXTAS in carriers of fully silenced *FMR1* alleles has led to the hypothesis that FXTAS is the result of a toxic gain-of-function of the *FMR1* mRNA itself (Hagerman and Hagerman 2004a; Hagerman et al. 2001). This hypothesis is based on a precedent set by the myotonic dystrophies, DM1 and DM2, in which expression of mutant RNAs containing hundreds to thousands of CUG (DM1) or CCUG (DM2) repeats accumulate in nuclear RNA aggregates that sequester specific RNA-binding proteins (the Muscleblind-like proteins). Depletion of the free pool of MBNL proteins leads to specific RNA metabolisms changes, which ultimately results in the symptoms of myotonic dystrophy (Nelson et al. 2013; Ranum and Cooper 2006).

Extending this RNA gain-of-function model to FXTAS predicts that the mutant *FMR1* RNA containing expanded CGG repeats may sequester specific proteins

resulting in loss of their normal functions, which would ultimately cause the symptoms of FXTAS (Hagerman and Hagerman 2004a). Consistent with this hypothesis, Iwahashi and collaborators identified more than 20 proteins from inclusions purified from brains of FXTAS patients (Iwahashi et al. 2006). Among these, two RNA-binding proteins were of special interest, hnRNP A2/B1, that is mutated in families with inherited degeneration affecting muscle, brain, bone and motor neurons (Kim et al. 2013), and MBNL1, the RNA-binding protein that is sequestered in myotonic dystrophy (Kanadia et al. 2003; Miller et al. 2000). However, a role for MBNL1 in FXTAS appears unlikely, since no genetic interaction between MBNL1 and CGG-mediated neurodegeneration was observed in fly model of FXTAS (Sofola et al. 2007), and no misregulation of splicing events regulated by MBNL1 were observed in brain samples from FXTAS patients (Sellier et al. 2010).

Association of hnRNP A2/B1 to RNA containing expanded CGG repeats was confirmed by several independent analyses (Jin et al. 2007; Sellier et al. 2010; Sofola et al. 2007). Specifically, the interaction of hnRNP A2/B1 with RNA containing expanded CGG repeats was observed in cytoplasmic cerebellar lysates (Sofola et al. 2007), and the importance of the titration of the cytoplasmic pool of hnRNP A2/B1 by expanded CGG repeats was demonstrated by the impaired dendritic delivery of the BC1 RNA, a known target of hnRNP A2/B1 (Muslimov et al. 2011). In contrast, nuclear hnRNP A2/B1 exhibited little binding to CGG RNA (Sofola et al. 2007), and alternative splicing regulated by nuclear hnRNP A2/B1 were not altered in brain samples of FXTAS patients (Sellier et al. 2010). These data suggest that some modifications of hnRNP A2/B1, either in the nucleus or in the cytoplasm, may modify the ability of hnRNP A2/B1 to bind to CGG RNA, so that expanded CGG repeats may recruit and deplete the cytoplasmic pool of hnRNP A2/B1, but not the nuclear pool of hnRNP A2/B1. In addition, the ability of hnRNP A proteins to unfold RNA structures formed by expanded CGG repeats (Khateb et al. 2004; Ofer et al. 2009) raises the interesting hypothesis that hnRNP A2/B1 may also act as an RNA chaperone that destabilizes the RNA structures formed by expanded CGG repeats.

hnRNP A2/B1 recruits other proteins, such as the CUGBP1 RNA-binding protein, to RNA containing expanded CGG repeats (Sofola et al. 2007). Overexpression of either hnRNP A2/B1 or CUGBP1 rescues the neurodegeneration in a *Drosophila* expressing 90 CGG repeats, highlighting the potential importance of hnRNP A2/B1 and CUGBP1 to FXTAS pathology (Jin et al. 2007; Sofola et al. 2007). Similarly, hnRNP A2/B1 interacts with the heterochromatin protein 1 (HP1) to silence the expression of specific *Drosophila* retrotransposons, such as *gypsy* or *copia*, and the titration of hnRNP A2/B1 by expanded CGG repeats results in increased expression of these retrotransposons in fly model of FXTAS (Tan et al. 2012). Knockdown of *gypsy* RNA expression, but not of *copia*, suppress the toxicity induced by expanded CGG repeats in *Drosophila* (Tan et al. 2012). Whether the expression of retrotransposons is altered in FXTAS patients remains to be determined, but it is interesting to note that increased levels of 5-hydroxymethylcytosine (5hmc) have been identified in repetitive elements DNA in a mouse model of FXTAS (Yao et al. 2014).

In addition to hnRNP A2/B1, proteomic analyses revealed that SAM68, a splicing regulator encoded by the *KHDRBS1* gene, was also found in CGG RNA aggregates (Sellier et al. 2010). However, overexpression of SAM68 was not suf-

ficient to rescue neuronal cell death induced by expression of expanded CGG repeats. Similarly to hnRNP A2/B1 that recruits CUGBP1 through protein–protein interactions, SAM68 did not bind directly to CGG repeats and recruitment of SAM68 within CGG RNA aggregates occurred in *trans*.

Sellier and collaborators (2013) found that DROSHA-DGCR8, the enzymatic complex that processes pri-microRNAs into pre-miRNAs, associates directly with expanded CGG repeats. Sequestration of DROSHA-DGCR8 within CGG RNA aggregates resulted in reduced processing of pri-miRNAs in neuronal cells expressing expanded CGG repeats, and overexpression of DGCR8 rescued neuronal cell death induced by expression of CGG repeats (Sellier et al. 2013). DROSHA-DGCR8 also recruits SAM68 to CGG repeats. Other studies also demonstrated alteration of the expression of specific miRNAs in blood samples of FXTAS patients and in *Drosophila* expressing CGG repeat (Alvarez-Mora et al. 2013; Tan et al. 2012). These data suggest that misregulation of the processing and/or of the expression of miRNA may be of importance for the pathogenicity of FXTAS.

Proteomic analysis performed by Jin and collaborators (2007) showed that Purα (purine-rich binding protein α) binds to RNA containing expanded CGG repeats. Purα is a single-stranded cytoplasmic DNA- and RNA-binding protein that has been implicated in many biological processes, including RNA transport and translation. Importantly, overexpression of Purα rescued neurodegeneration in a *Drosophila* model of FXTAS (Jin et al. 2007). However, the presence of Purα within nuclear aggregates in FXTAS brain samples was not consistently observed. Jin et al. (2007) found Purα in cytoplasmic inclusions in *Drosophila* expressing 90 CGG repeats. In contrast, Purα was not identified within the intranuclear inclusions in brain sections of mouse premutation models, or in hippocampal or cortical brain sections derived from patients with FXTAS (Galloway et al. 2014; Iwahashi et al. 2006; Sellier et al. 2013). These results indicate that composition of the inclusions may vary from one model organism to the other.

As with hnRNP A2/B1 that recruits CUGBP1, Purα recruits Rm62, the *Drosophila* ortholog of the RNA helicase P68/DDX5 (Qurashi et al. 2011). Overexpression of Rm62 rescued neurodegeneration in flies expressing 90 CGG repeats, highlighting the potential importance of P68/DDX5 to FXTAS pathology (Qurashi et al. 2011). Of interest, the RNA helicases P68/DDX5 and DDX6 have been recently reported to be involved in the unfolding of expanded CUG repeats in myotonic dystrophy (Laurent et al. 2012; Pettersson et al. 2014). These data raise the hypothesis that regulating the RNA structures of CUG expanded repeats in DM, or of CGG repeats in FXTAS may be of therapeutic interest (Disney et al. 2012; Tran et al. 2014; Yang et al. 2015).

Finally, simultaneous studies by Peter Todd and David Nelson's groups demonstrated that overexpression of TDP-43, encoded by the *TARDBP* gene, suppresses the neurodegeneration induced by expanded CGG repeats in *Drosophila* (Galloway et al. 2014; He et al. 2014). Interestingly, the expression of *Tardbp* is down-regulated in Purkinje neurons of a mouse model of FXTAS expressing the 5′UTR of *FMR1* with 90 CGG repeats under the Pcp2 promoter (Galloway and Nelson 2009). TDP-43 is of special interest for neurodegenerative diseases since protein inclusions of TDP-43 in neurons is a histopathological hallmark of amyotrophic lateral sclerosis (ALS) (Neumann et al. 2006), and mutation of *TARDBP* leads to amyotrophic lateral sclero-

sis and frontotemporal dementia (ALS-FTD) (Kunst and Warren 1994). Interestingly, TDP-43 is not found within the RNA aggregates of CGG repeats, but requires the hnRNP A2/B1 homologues Hrb87F and Hrb98DE to modulate the CGG repeat triggered toxicity (He et al. 2014). Overall, these observations suggest that mutant *FMR1* RNA containing expanded CGG repeats could be pathogenic by sequestering specific RNA-binding proteins, resulting in loss of their normal functions, ultimately leading to neuronal cell dysfunction and death. In that aspect, it remains to be determined whether expression of Purα, hnRNP A2/B1, P68/DDX5, DROSHA-DGCR8, CUGBP1, or TDP-43 rescues any phenotype in mouse models expressing expanded CGG repeats. Such functional rescue experiments will be instrumental to determine the importance of these candidate RNA-binding proteins to FXTAS pathology.

Inclusion Formation

The presence of eosinophilic intranuclear inclusions, broadly distributed throughout the brain and brainstem of affected individuals (Greco et al. 2002, 2006; Tassone et al. 2004) represent the neuropathological hallmark of FXTAS. These inclusions are present as single, spherical (~2–5 μm of diameter) particles that are found exclusively within the nuclei. Inclusions have been identified in both neurons and astrocytes throughout the cerebrum and the brainstem, including cells of the ependymal and sub-ependymal layers (Tassone et al. 2004). They are most numerous in the hippocampus and are rarely seen in Purkinje cells of the cerebellum (Greco et al. 2002, 2006). Outside of the CNS, rare inclusions have been observed in both the pineal and the posterior and anterior pituitary glands but also in the pancreas, in the heart, in the thyroid and adrenal glands, in the Leydig cells of the testis, in ganglia and in periadrenal fat tissue (Greco et al. 2007; Hunsaker et al. 2011). Inclusions have also been observed in postmortem brain tissue of females with FXTAS (Hagerman et al. 2004; Tassone et al. 2012b).

Pioneering work from Hagerman and Tassone's laboratories demonstrated that these inclusions are ubiquitin-positive and contain various chaperone proteins such as Hsp27, Hsp70, and αB-crystallin, but are negative for tau proteins, α-synuclein, or polyglutamine (Greco et al. 2002; Iwahashi et al. 2006). Importantly, these inclusions also stain positive for the *FMR1* RNA messenger as well as for its polyglycine RAN-translated product (Buijsen et al. 2014; Tassone et al. 2004; Todd et al. 2010) suggesting that expression of mutant *FMR1* mRNA may trigger inclusion formation. In support of this hypothesis, the formation of inclusions can be recapitulated in neuronal cell cultures using a minimal construct expressing an expansion of 99 CGG repeats embedded within the 5′UTR of *FMR1* (Arocena et al. 2005; Sellier et al. 2010; Todd et al. 2010). Of interest, plasmids harboring either no expanded CGG repeats or expanded CGG repeat but under an inactive promoter (no RNA) did not lead to inclusion formation. Also, intranuclear inclusions formed even when the portion encoding FMRP was deleted (Arocena et al. 2005; Todd et al. 2010). Thus, expression of the 5′UTR of *FMR1* containing a CGG repeat expansion is necessary and sufficient for the formation of these inclusions.

These observations in neuronal cell cultures are consistent with the work of Jin et al. (2003), who showed that expression in *Drosophila* of a construct containing the 5′UTR of *FMR1* with 90 CGG repeat induces the formation of cytoplasmic ubiquitin-positive inclusions associated with neurodegeneration and eye pathology (Jin et al. 2003). Similarly, a knock-in (KI) mouse model, in which the endogenous eight CGG repeats of the murine *Fmr1* were replaced with an expansion containing ~100 CGG repeats of human origin, demonstrated ubiquitin-positive nuclear inclusions and mild neuromotor and behavioral disturbances (Brouwer et al. 2008; Van Dam et al. 2005; Willemsen et al. 2003). A result confirmed by recent mouse models where heterologous expression of the sole 5′UTR of *FMR1* containing expanded CGG repeats under strong chimeric promoters is sufficient to cause cellular toxicity, despite the presence of normal *Fmr1* alleles and unaltered expression of Fmrp (Hashem et al. 2009; Hukema et al. 2014). These animal models demonstrate that the expression of *FMR1* mRNA containing expanded CGG repeats is both necessary and sufficient to cause the pathological features of human FXTAS (Berman et al. 2014).

RAN Translation Produces a Polyglycine-Containing Protein in FXTAS

What drives inclusion formation in FXTAS was unclear until recently. The working hypothesis in the field was that the CGG RNA repeats were serving as a nidus for inclusion formation by nucleating together a set of RNA-binding proteins. However, while some inclusions in brain samples of FXTAS patients contain mutant *FMR1* RNA with expanded CGG repeats (Tassone et al. 2004), mouse models in which express expanded CGG repeats show numerous ubiquitin-positive inclusions but only rarely RNA aggregates, suggesting that other factors might trigger inclusion formation (Sellier et al. 2013). Moreover, the inclusions observed in FXTAS are ubiquitin positive, which is a characteristic not commonly observed in other RNA-dominant disorders such as Myotonic Dystrophy.

A hint for how nucleotide repeats in the 5′UTR of a messenger RNA might trigger inclusion formation came with the discovery of repeat associated non-AUG (RAN) translation by Laura Ranum and colleagues (Zu et al. 2011). They found that expression of either CAG in cells outside the context of an AUG-initiated open reading frame were capable of supporting translational initiation and production of homopolymeric proteins. Surprisingly, this RAN translation occurred in all three possible reading frames to produce polyglutamine, polyalanine, and polyserine containing proteins. Initiation was largely independent of the surrounding sequence context but was influenced by the cell type in which the repeats were expressed and was somewhat different for different repeat frames. They then established that these alternative "RAN" products accumulated in tissues in animal models of Spinocerebellar Ataxia type 8 and in lymphoblasts from patients with DM1 (where a CAG repeat is expressed as an antisense transcript). Since these initial observations, RAN translation has been reported in association with CUG repeats (as seen in DM1) and with both GGGGCC and CCCCGG repeat expansions in C9 or f72

that cause ALS and Frontotemporal Dementia (Ash et al. 2013; Gendron et al. 2013; Mori et al. 2013a, b; Zu et al. 2011, 2013).

Todd and colleagues (2013) independently discovered that placement of CGG repeats upstream of a fluorescent reporter protein (GFP) in certain reading frames led to aggregation of GFP in transfected cells and in *Drosophila*. This aggregation was associated with production of a higher molecular weight species that resulted from translational initiation within the 5′UTR just above the CGG repeat. They were able to demonstrate by mass spectroscopy and biochemical analysis that this unconventional translational initiation (CGG RAN translation) occurred at one of several "near" AUG codons (one nucleotide different from AUG) to produce products in two reading frames, producing a polyglycine containing protein (FMRpolyG) and a polyalanine containing protein (FMRpolyA) (Todd et al. 2013). Production of FMRpolyG was confirmed in patients by use of antibodies generated against the predicted C terminus of this novel protein. The FMRpolyG protein was found in a fraction of ubiquitinated inclusions in brain autopsy samples from patients (Todd et al. 2013). Recent studies with a second set of antibodies covering different N-terminal and C-terminal epitopes revealed that most of the ubiquitin-positive inclusions observed in FXTAS brain sections are also FMRpolyG positive (Buijsen et al. 2014).

A role for FMRpolyG in disease pathogenesis is supported by work in *Drosophila.* In both transfected cells and in flies, it was demonstrated that moving the repeat to the 3′UTR or placing a stop codon just above the repeat prevented FMRpolyG production and suppressed CGG repeat associated toxicity. In contrast, placing an AUG start codon above the repeat-enhanced FMRpolyG production and led to greater lethality in flies and cells. However, the mechanism by which FMRpolyG induces toxicity is still unclear. Impairment of the ubiquitin proteasome system (UPS) enhances CGG repeat associated toxicity (Handa et al. 2005; Oh et al. 2015; Todd et al. 2013) and expression of chaperone proteins such as HSP-70 suppresses CGG repeat associated toxicity in flies (Jin et al. 2003). This genetic interaction between UPS impairment and CGG toxicity is largely driven by RAN translation (Oh et al. 2015). Moreover, RAN translation is capable of triggering UPS impairment in transfected cells (Oh et al. 2015). Together these studies suggest that failures in protein quality control are likely one method by which RAN translation cause toxicity, but future work will be needed to define how this unusual process contributes to FXTAS pathogenesis.

Disruption of Lamin A/C Architecture

Among the various proteins that Iwahashi and collaborators identified within the inclusions in brain samples of FXTAS patients (Iwahashi et al. 2006), the lamin A/C proteins are of special interest. Lamin proteins constitute a matrix of protein that is located next to the inner nuclear membrane and that is involved in nuclear stability, chromatin structure, and gene expression. Mutations in the *LMNA* gene, which encodes both lamin A and C splicing forms, are associated with several diseases

called laminopathies, including limb girdle and Emery–Dreifuss muscular dystrophies, dilated cardiomyopathy, lipodystrophy, Charcot–Marie–Tooth disease and Hutchinson–Gilford progeria syndrome. Interestingly, nuclear organization of lamin A/C is disrupted in MEF of knock-in mice expressing ~200 CGG repeats, as well as in skin fibroblasts of FXTAS patients with loss of the typical architecture of lamin A/C within the nuclear ring (Garcia-Arocena et al. 2010). These alterations of the lamin A/C structure can be recapitulated in cell cultures upon transfection of a minimal construct expressing expanded CGG repeats embedded within the 5′UTR of *FMR1* (Arocena et al. 2005; Hoem et al. 2011; Sellier et al. 2010). Also, the expression of *Lmna* mRNA is decreased in Purkinje neurons of a mouse model of FXTAS expressing 90 CGG repeats under the Pcp2 promoter (Galloway et al. 2014). In contrast, the expression of *LMNA* mRNA is increased while the amounts of soluble protein of Lamin A and C are slightly reduced in brain samples of FXTAS patients (Garcia-Arocena et al. 2010). Overall, these results suggest that lamin A/C architecture and expression are altered in FXTAS; however, it remains to determine the molecular mechanism leading to these alterations. It is therefore intriguing that recent data on GGGGCC repeat expansions in *C9orf72* which cause ALS and FTD are associated with changes in nuclear envelope architecture and nuclear pore complex function (Freibaum et al. 2015; Jovicic et al. 2015; Zhang et al. 2015). Thus, the pathological consequences of lamin alterations in FXTAS are an exciting area of research in need of further exploration.

Mitochondrial Dysfunction

Mitochondrial biogenesis plays a central role in neurogenesis and neuroplasticity and several studies have demonstrated significant mitochondrial dysfunction in premutation carriers (Kaplan et al. 2012; Napoli et al. 2011; Ross-Inta et al. 2010), both with and without fragile X-associated tremor ataxia syndrome (FXTAS). Specifically, altered biochemical characteristics pointing to a lower ATP production, including decreased NAD- and FAD-linked oxygen uptake rates, decreased mitochondrial protein expression, increased oxidative/nitrative stress, and altered zinc bioavailability were demonstrated in both cultured dermal fibroblasts and brain samples from premutation carriers with and without FXTAS, within a wide range of CGG repeats (Napoli et al. 2011; Ross-Inta et al. 2010). Kaplan et al. (2012) observed fewer mitochondria and greatly reduced mitochondria mobility in hippocampal neuronal culture from preCGG KI mice, expressing low FMRP levels and higher *Fmr1* mRNA than that measured in wild type during the early stages of development. In addition, preCGG neurons presented with abnormal metabolic function including higher rates of basal oxygen consumption, ATP production, and proton leak. The authors suggested that deficits in mitochondrial trafficking and metabolic function could play a role in the early pathophysiology observed in premutation carriers and also potentially contribute to the risk of developing FXTAS.

In addition, an altered Ca^{2+} regulation was reported in both neuronal cultures from the premutation mouse model (*Fmr1* preCGG mouse) (Cao et al. 2012) but also in iPSC derived from premutation carriers (Liu et al. 2012). The impaired Ca^{2+} regulation was associated with alteration of the most abundant excitatory neurotransmitter in the vertebrate CNS, the Glutammate, with a decrease in the expression of GLT-1 and GLAST Glutamate uptake and of their expression levels in astrocytes cultures from preCGG mice and in postmortem cerebellum of PM carriers with FXTAS compared with age-matched controls. Higher CGG repeat number had the greatest reductions in both proteins (Cao et al. 2013; Pretto et al. 2014b). Abnormal clustered burst (CB) firing electrical activity and abnormal patterns of synchronized calcium oscillations in the cytosol were also observed with the premutation mouse neurons, which, interestingly, were reversed with mGluR5 antagonists or the GABAA receptor positive modulator allopregnanolone (Cao et al. 2012) making it a potential candidate for a beneficial therapeutic approach in FXTAS.

Finally, shorter telomeres have been observed in a number of neurodegenerative conditions including Alzheimer disease, cell senescence, and also in Down syndrome (Jenkins et al. 2006; Panossian et al. 2003). The role of telomeres includes protection against degeneration; they also appear to be essential for chromosomal stability and could lead to cell senescence. Telomere shortening has also been observed in a small sample of male carriers of the premutation (Jenkins et al. 2008) with FXTAS and with or without dementia. However, further studies are warranted to establish if telomere shortening could be considered a biomarker for the cellular dysfunction observed in FXTAS.

The above observations suggest that many triggering events can be associated with CGG expansion leading to cellular dysregulation and dysfunction and ultimately, potentially, to neurodegeneration and *FMR1*-associated disorders.

Summary

Individuals with expanded repeat of 55–200 CGG repeats are at risk to develop the late onset disease FXTAS, which might be the most common progressing neurological disorder associated with a single gene defect in males. FXTAS is likely to be the consequence of the increased expression of mutant *FMR1* mRNA containing expanded (55–200) CGG repeats. Despite recent and important advances, the molecular mechanisms that lead to both inclusion formation and cell toxicity in FXTAS remain to be clarified. Several nonexclusive models for RNA-triggered pathogenesis in FXTAS can be hypothesized (summarized in Fig. 6.2). First, it is possible, as in the case of myotonic dystrophy, that expanded CGG repeats toxicity, derives from sequestration of CGG RNA-binding proteins, which would limit the availability of those proteins to carry out their normal cellular functions. Alternatively, interactions between yet to be defined proteins and expanded CGG repeats could lead to activation of downstream signaling cascades potentially harmful for the cell. A second mechanism involves noncanonical translation of the

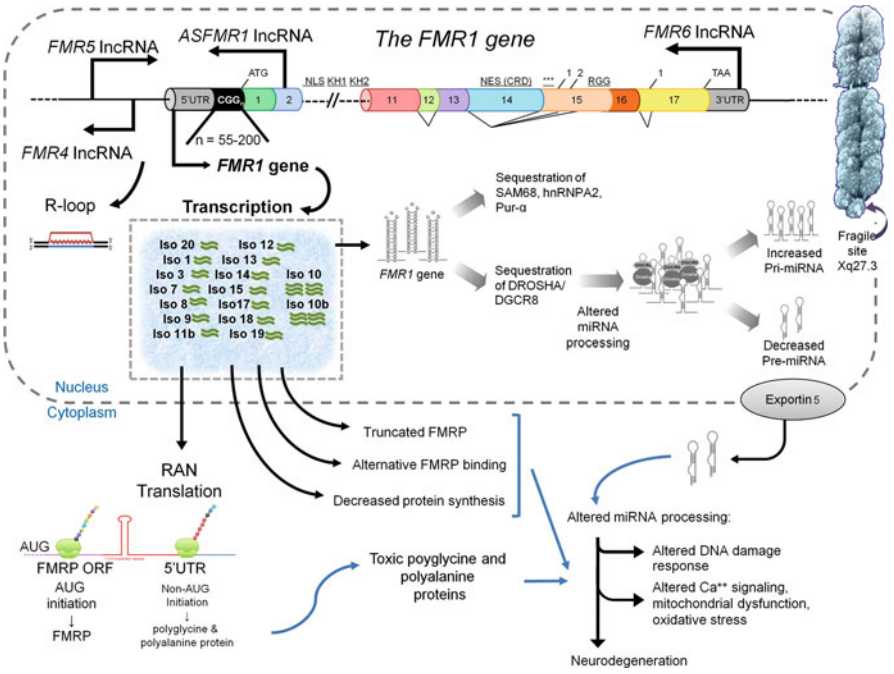

Fig. 6.2 Diagram showing the molecular alterations of premutation expanded alleles including increased FMR1 mRNA expression levels, R-Loop formation, sequestration of CGG binding proteins, miRNA dysregulation and RAN translation

expanded CGG repeats in a small protein composed of polyglycine, which is prone to aggregation. However, whether and how FMRpolyG alters neuronal function in mammalian systems requires further elucidation. Finally, mild decreased expression of the FMRP protein may contribute to FXTAS pathogenesis.

In conclusion, contributions to pathology from multiple pathogenic mechanisms may converge to explain the great variability in clinical presentations of FXTAS. Thus, more studies are warranted to improve our understanding of this multifaceted disorder and to design and develop appropriate targeted therapeutic approaches.

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Chapter 7
Genotype/Phenotype Relationships in FXTAS

Emily G. Allen, Maureen A. Leehey, Flora Tassone, and Stephanie Sherman

Abstract In this chapter we explore the effects of molecular measures of the *FMR1* gene on clinical, cognitive, radiological, and pathological phenotypes associated with fragile X-associated tremor/ataxia syndrome (FXTAS). In addition to reviewing the FXTAS phenotype, we will also present methods that have been developed for quantifying severity of FXTAS symptoms, including development and use of the FXTAS rating scale, the neuropathy screening scale, and use of the CATSYS system to assess early, prodromal symptoms. In our review of phenotypic features, we focus primarily on studies in which findings were correlated with *FMR1* molecular measures. The phenotypes considered include (1) clinical neurological measures, (2) cognitive measures with a focus on executive function, (3) psychiatric phenotypes, (4) radiological outcomes, and (5) pathological measures. Because women are more mildly affected than men and may, in fact, have a different presentation, we will review FXTAS among men and women separately. Overall, our goal in this chapter is to begin to define alleles that are "at risk" for FXTAS. Based on this review, it is clear that there is a strong correlation of the increasing risk for FXTAS with increasing repeat size. However, much work needs to be done to further define the properties of the CGG repeat sequence that contribute to increased risk and severity of symptoms of FXTAS among men and women.

Keywords FXTAS • *FMR1* • Premutation • Genotype/phenotype

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Introduction

In this chapter we explore the effects of molecular measures of the *FMR1* gene on clinical, cognitive, radiological, and pathological phenotypes associated with fragile X-associated tremor/ataxia syndrome (FXTAS). Most studies have reported association studies between CGG repeat length and symptoms of FXTAS. Further, it is now well established that *FMR1* mRNA levels increase with repeat length (Allen et al. 2004; Garcia-Alegria et al. 2007; Kenneson et al. 2001; Tassone et al. 2000) and FMRP levels decrease (Feng et al. 1995; Kenneson et al. 2001; Primerano et al. 2002) and that the repeat tract in the mRNA has a toxic effect on neuronal cells (Arocena et al. 2005), and several reports have also examined this important molecular outcome. For females, we will review the additional assessment of the X-chromosome activation ratio (the percentage of cells that carry the normal *FMR1* allele on the active X-chromosome), an important consideration for expression of FXTAS, as a condition mediated by an X-linked gene. In addition to reviewing the FXTAS phenotype, we will also present methods that have been developed for quantifying severity of FXTAS symptoms, including development and use of the FXTAS rating scale, the neuropathy screening scale, and use of the CATSYS system to assess early, prodromal symptoms. Such methods are important when examining genotype/phenotype correlations. In each of the following sections, we will begin with a review of the major features of the phenotype being considered, discuss methods used to measure the severity of the phenotype, and then summarize studies that have correlated the *FMR1* genotype or other molecular measures with phenotype. In our review of phenotypic features, we focus primarily on studies in which findings were correlated with *FMR1* molecular measures. More thorough reviews of the specific phenotypes can be found in Chaps. 1 and 3–5. The phenotypes considered include (1) clinical neurological measures, (2) cognitive measures with a focus on executive function, (3) psychiatric phenotypes, (4) radiological outcomes, and (5) pathological measures. Because women are more mildly affected than men and may, in fact, have a different presentation, we will review FXTAS among men and women separately.

Overall, our goal in this chapter is to begin to define alleles that are "at risk" for FXTAS. To date, the definition of premutation alleles (55–200 repeats) has been based only on the risk for expansion of the premutation to the full mutation (i.e., the instability of the repeat sequence) when transmitted from parent to child. Although this is important when assessing the risk for fragile X syndrome (FXS) in the next generation, it provides only a crude estimate of the risk for severe symptoms of FXTAS. Based on this review, it is clear that there is a strong correlation of the increasing risk for FXTAS with increasing repeat size. However, much work needs to be done to further define the properties of the CGG repeat sequence that contribute to increased risk and severity of symptoms of FXTAS among men and women.

Genotype/Phenotype Correlation Among Men with the Premutation

Clinical Neurological Phenotype

Overview

The initial report of FXTAS presented five elderly men with a premutation who experienced a progressive action tremor, ataxia, and executive functioning deficits, had generalized brain atrophy, and showed elevated *FMR1* mRNA levels (Hagerman et al. 2001). Upon further examination of additional premutation carrier men with symptoms of FXTAS ($N=20$), diagnostic criteria were established based on clinical presentation of motor and cognitive symptoms and radiological findings as outlined in Chap. 1. Specifically, criteria were based on the presence of a combination of "major" and "minor" symptoms as described in Jacquemont et al. (2003). "Definite FXTAS" includes individuals who show the major radiological symptom (middle cerebellar peduncles lesions) and at least one major clinical symptom (intentional tremor or gait ataxia); "probable FXTAS" includes either individuals with the major radiological symptom and one of the minor clinical symptoms (parkinsonism, memory, or executive function deficit) or those with both major clinical symptoms; "possible" FXTAS includes individuals that show a minor radiological symptom (cerebral white matter hyperintensity or cerebral cortical atrophy) and one of the major clinical symptoms (Jacquemont et al. 2003). For the 20 patients in this cross-sectional study, the average age of onset of tremor was 63 ± 8 years, for gait instability was 63 ± 6 years, for memory difficulties was 63 ± 6 years, for urinary incontinence was 64 ± 6 years, and for death was 74 ± 6 years (Jacquemont et al. 2003). Based on self-report in a larger cohort of men with FXTAS, the penetrance of tremor and ataxia was estimated (Jacquemont et al. 2004). The percentage of individuals who reported symptoms of ataxia ranged from 17 % in men with the premutation between the ages of 50 and 59 to 100 % among men over the age of 80. Similarly, 17 % of men with the premutation aged 50–59 self-reported symptoms of tremor, and the frequency increased to 75 % of those over the age of 80. Juncos et al. (2011) suggested that the current diagnostic criteria was too heavily weighted by the MRI finding of the MCP sign, which may be a function of symptom progression. In 2012, Apartis et al. suggested adding additional measures to the diagnostic criteria: corpus callosum splenium hyperintensity and peripheral neuropathy (Apartis et al. 2012).

Clinical severity of FXTAS has been estimated by using an empirical staging system, which incorporates the motor signs of FXTAS. The system gives an indication of the impact of motor aspects of the disease on activities of daily living (ADLs). The stages range from 0 to 6, where stage 0 describes normal functions; stage 1 indicates subtle or questionable signs such as subtle tremor or mild balance problems and no interference with ADLs; stage 2 indicates clear tremor and/or balance problems and minor interference with ADLs; stage 3 indicates moderate

tremor and/or balance problems and occasional falls and significant interference with ADLs; stage 4 indicates severe tremor and/or balance problems with at least intermittent use of a cane or a walker; stage 5 involves the use of a wheelchair on a daily basis; and in stage 6, subjects are bedridden.

Once criteria for diagnosis of FXTAS were established, investigators screened various populations of patients with movement disorders to determine the frequency of those who carried the premutation allele. Details of the numerous screening studies reported to date can be found in Chap. 2.

After the original descriptive criteria and FXTAS staging system were defined, it became clear there was a need for more quantitative measures of disease severity, and investigators have begun to develop specific methods to better evaluate symptoms and examine findings and their severity. We will first review the FXTAS and neuropathy rating scales, then a quantitative method to evaluate motor symptoms, and finally other tools including nerve conduction studies to better define the clinical phenotype.

FXTAS Rating Scale: Quantifying the Severity of Motor Signs of FXTAS

The FXTAS rating scale was developed to determine the severity of motor signs of FXTAS, tremor, ataxia, and parkinsonism. Initially the scale was a combination of three separate measures: the Clinical Rating Scale for Tremor (CRST) (Fahn and Tolosa 1987), the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al. 1997), and the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn and Tolosa 1987). Using this FXTAS rating scale in a blinded videotape study, Berry-Kravis et al. (2003) found group differences between men with the premutation ($N=7$) and all other groups ($N=30$) on each of the component scales. On the CRST, men with the premutation showed significantly increased levels of rest, postural, and kinetic tremor. For both the ICARS and the UPDRS, they showed increased scores for tremor and limb ataxia (Berry-Kravis et al. 2003).

Subsequently the FXTAS rating scale has undergone clinimetric testing with elimination and modification of some items and addition of a tandem test (Huntington Study Group 1996). The resultant scale has 44 items and a total score of 226. The tremor sub-domain (score range 0–53) assesses action and postural tremor, drawing, and handwriting. The ataxia sub-domain (0–73) assesses posture and gait, limb ataxia, dysarthria, and oculomotor disturbances. The parkinsonism sub-domain (0–100) assesses bradykinesia, gait and balance, rest tremor, and rigidity. Use of this FXTAS rating scale in a cross-sectional study effectively discriminated between the degree of major motor signs in male carriers ($n=54$) vs. controls ($n=51$) (Fig. 7.1) (Leehey et al. 2008).

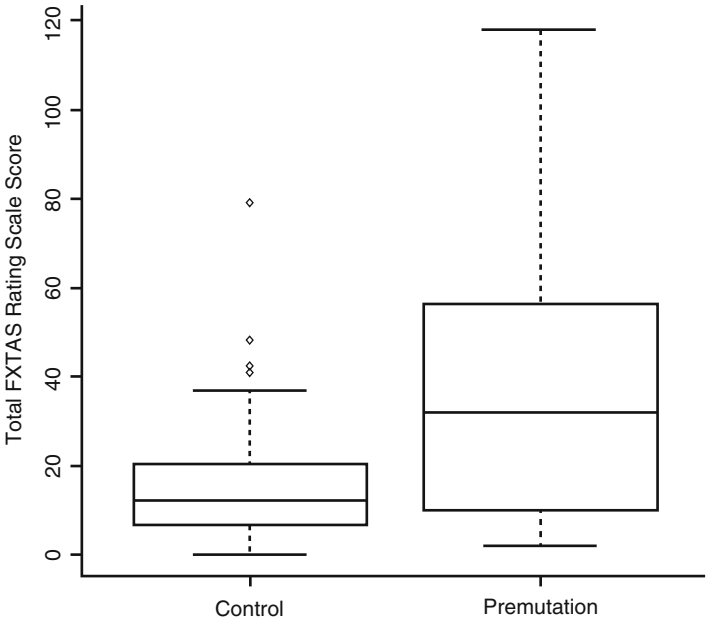

Fig. 7.1 Box plot showing the distribution of total fragile X-associated tremor/ataxia syndrome rating scale scores (score range 0–226) in male controls vs. carriers. Taken from Leehey et al. (2008)

Neuropathy Rating Scale

Berry-Kravis et al. (2007) went further to ask if the neuropathic signs that had been described in previous studies were related to tremor or ataxia as assessed by the FXTAS rating scale and/or related to CGG repeat length (see below). The neuropathic assessment was based on abstraction from neurological examination medical records. The scale included the evaluation of findings pertaining to ankle reflexes and vibratory sensation in the feet. These were found to be commonly available in the records and conducted in a standardized way. The rating score was quantitative with 0 indicating no signs of neuropathy and up to a maximum (total) impairment score of 8 (4 for reflexes and 4 for vibration) if there was full reflex and vibratory loss bilaterally in the distal lower extremities. The study included 49 men with the premutation and 46 noncarrier men.

Overall, the men in the premutation carrier group had significantly higher mean scores on the total neuropathy screening scale ($p=0.0014$), the vibration score alone ($p=0.015$), and the reflex score alone ($p=0.0014$) compared with the control group. These findings suggest that greater impairment of both distal vibratory sense and reflexes occurs in men with the premutation.

Both the FXTAS and the neuropathy rating scales were available on 49 premutation carriers. There was no correlation between any of the neuropathy scores and the

tremor subscores; however, there was a significant correlation (age-adjusted) between the total neuropathy scores and the ataxia subscore (partial correlation coefficient = 0.339; $p = 0.018$). This finding was primarily driven by the association between the reflex score and the ataxia subscore (partial correlation coefficient = 0.362; $p = 0.012$). The authors concluded that the observed neuropathic signs are increased in male carriers of the premutation and that neuropathic signs are more prominent in men with more severe ataxia.

CATSYS: Quantifying Early Motor Symptoms of FXTAS

The CATSYS 2000 (www.catsys.dk) is a portable testing system that is used to measure coordination ability, reaction time, tremor, and postural stability. This system has been tested in two large studies to identify early symptoms of FXTAS in men with the premutation. First, Allen et al. (2008) presented results on 89 men for whom FXTAS status was unknown: 62 with ≥70 repeats (high-repeat allele carriers) and 27 with <70 repeats (low-repeat allele carriers). Aguilar et al. (2008) presented data on 46 males: 16 premutation carriers with FXTAS, 16 premutation carriers who did not have FXTAS, and 14 controls.

Ataxia is tested by the CATSYS system by using a force plate attachment. Trials are done with eyes open and eyes closed. Both studies detected differences for ataxia between groups in their study populations. In Allen et al. (2008), ataxia was detected significantly more often among the high-repeat allele carriers that were between the ages of 50 and 70 for both the eyes open trials and the eyes closed trials. Aguilar et al. (2008) detected a significant difference for the FXTAS group compared to controls for the eyes open and eyes closed trials. Differences were also seen between the FXTAS groups compared to the non-FXTAS premutation carriers for the eyes open trial (Aguilar et al. 2008).

Tremor is tested by the CATSYS system using a stylus that contains an accelerometer that measures movement in two dimensions. Postural tremor has been tested using the CATSYS system in two ways. First, in both studies, individuals were asked to grasp the stylus like they would hold any writing utensil and steadily hold it at the abdomen level. Neither study was able to detect any significant differences between groups using this measure (Aguilar et al. 2008; Allen et al. 2008). In Allen et al. (2008), an additional measure of postural tremor was performed. Individuals were asked to stand with their arms extended perpendicular to their body. The stylus was affixed to the middle finger to detect any movement. Similar to the previous measure, no significant differences were detected between groups using this measure of postural tremor. Intention tremor has also been assessed using the CATSYS system. In Allen et al. (2008), individuals were asked to hold the stylus as they would a pencil and track the movement of a bar across the monitor of a computer without actually touching the screen. No significant differences were identified between groups; however, in a follow-up analysis of only those individuals who scored >1 standard deviation above the mean for a normative population for inten-

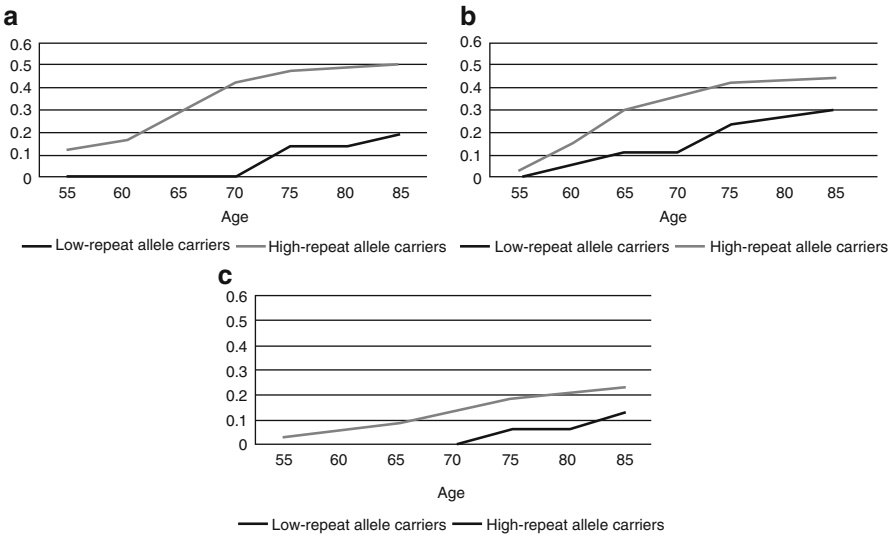

Fig. 7.2 Age-related prevalence of tremor (**a**), ataxia (**b**), and both tremor and ataxia (**c**) among male high-repeat allele carriers (70–199 repeats) and low-repeat allele carriers (<70 repeats). Presence of tremor and ataxia is defined as greater than 2 standard deviations above the mean score of a normative population. Taken from Allen et al. (2008)

tion tremor, a significant association was seen between tremor intensity and CGG repeat number (see below). In Aguilar et al. (2008), the task for intention tremor consisted of having individuals use the stylus to alternatively tap points on the computer screen. In this study, a significant difference was seen between premutation carrier males with FXTAS and controls.

Based on the CATSYS results, Allen et al. presented findings for the minimum estimates of age-related prevalence for tremor, ataxia, and both tremor and ataxia together (Fig. 7.2). For these estimates, the numerator was defined as the number of participants who were positive (defined as >2 standard deviations above the normative mean) for tremor (any of the three measures done) and/or ataxia at that age or earlier. The denominator included those same individuals plus those who had reached that age without developing tremor or ataxia. These measures were based on the presence of symptoms at the time of evaluation. They do not incorporate age of onset; thus the prevalence rates are minimum estimates for this population (Allen et al. 2008).

Nerve Conduction Studies: Tools to Identify Additional Symptoms of FXTAS

In a study of nerve conduction, 16 premutation carrier men with FXTAS, 11 without FXTAS, and 11 controls were evaluated in order to more accurately assess the neuropathy that had been previously reported in case studies (Soontarapornchai

et al. 2008). Five nerve conduction variables were studied: (1) the tibial compound muscle action potential (CMAP) amplitude; (2) the conduction velocity of CMAP; (3) the presence of abnormal temporal dispersion; (4) F-wave latency; and (5) the sural sensory nerve action potential (SNAP) amplitude. Men with FXTAS were significantly different from controls and asymptomatic premutation carriers for both tibial nerve conduction velocity and F-wave latency. Relative to controls only, FXTAS males showed smaller compound muscle action potential amplitudes and reduced sural nerve action potential amplitudes (Soontarapornchai et al. 2008).

The primary conclusion of this systematic evaluation was that men with FXTAS showed multiple aberrations of motor and sensory nerve conduction variables compared with those premutation carriers without FXTAS and with controls. Men with FXTAS compared to controls exhibited significantly reduced tibial CMAP and sural SNAP amplitudes, slow conduction velocity of CMAP, and prolonged F-wave latency. The authors point out that several non-FXTAS carriers had temporal dispersion and prolonged F-wave latency. This finding indicates that such nerve conduction abnormalities may be early signs of later neurological symptoms of FXTAS. Further longitudinal studies using nerve conduction studies are needed to test this hypothesis.

Genotype/Phenotype Correlations with Clinical Neurological Measures

The general conclusion from most studies that have examined the correlation of the *FMR1* genotype with the clinical neurological signs of FXTAS is that the presence and severity of clinical symptoms of FXTAS increase with increasing repeat size (Table 7.1). One of the first indications of this correlation came from the meta-analysis of the screening studies of patients with movement disorders (Jacquemont et al. 2006). Of the patients identified to be premutation carriers in the screening studies, the mean repeat size was 92 repeats (range 61–135) and 87 % had repeat sizes above 70 repeats. The average repeat size among men with premutation alleles identified through families with FXS was determined to be 83 repeats (range 55–107). Based on these findings and those from screening studies in the general population, the authors concluded that allele distribution of patients with FXTAS is significantly shifted toward larger repeat sizes, particularly those with expansions >70 repeats (Jacquemont et al. 2006).

Other studies that examined the correlation of clinical signs with repeat length and other molecular correlates have substantiated the finding from screening studies. With respect to motor symptoms, Tassone et al. (2007) identified a significant correlation between repeat length and age of onset for tremor ($p=0.001$), ataxia ($p=0.002$), or either ($p=0.0001$) phenotype among 93 men with the premutation (Fig. 7.3 and Table 7.1). Other molecular measures, including *FMR1* transcript levels and protein levels, did not show associations with age of onset of motor symptoms (Tassone et al. 2007). In a population of 50 subjects, Juncos et al. (2011) did

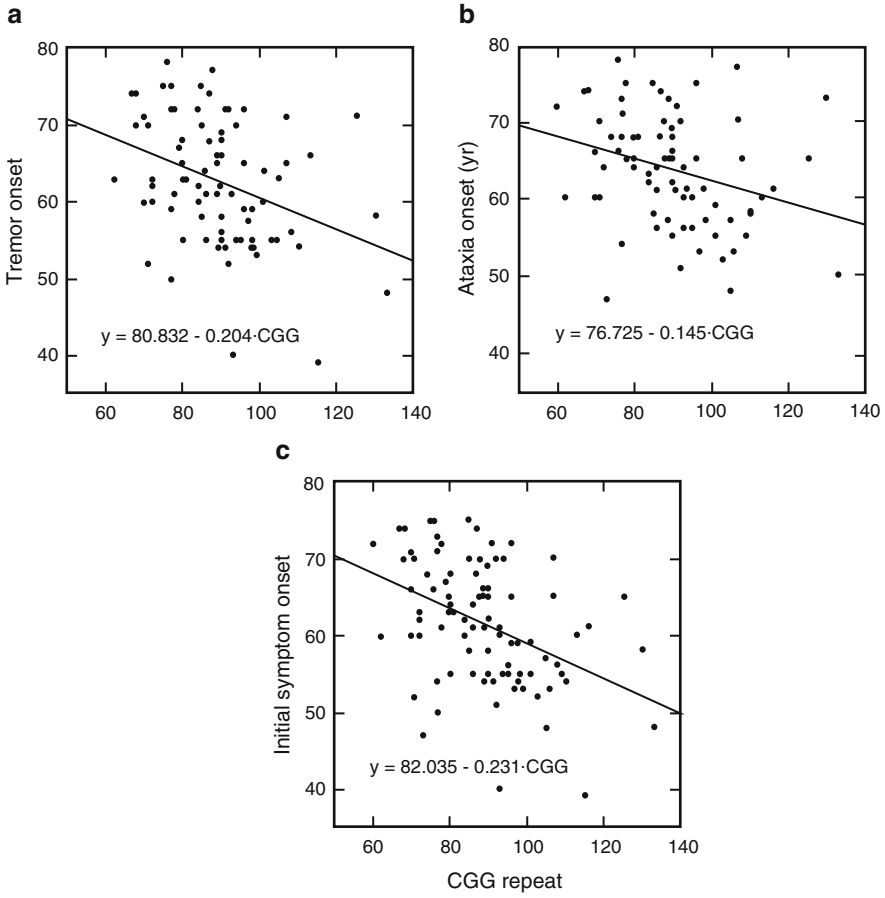

Fig. 7.3 Plots of the ages of onset of tremor (**a**), ataxia (**b**), and initial symptom (**c**), earlier of tremor or ataxia, when both are present as a function of the length of CGG repeat. Regression functions and *lines* are displayed for each dataset. Taken from Tassone et al. (2007)

not see a significant relationship with age of onset of motor symptoms, diagnostic category, or disease severity (as measured by the FXTAS rating scale or Modified Rankin score) and CGG repeat size.

Using the Total and Component subscores of the FXTAS rating scale, Leehey et al. (2008) also found significant support for a correlation between CGG repeat size and the overall severity of motor symptoms, as well as severity of symptoms in the tremor, ataxia, and parkinsonism subscores. There was no correlation with mRNA levels. Regression analysis, adjusting for age at examination, was used to identify these correlations among the full cohort of combined noncarriers and carriers: 54 premutation men and 51 control men as well as correlations among the premutation carrier group alone. Correlations adjusted by age and their associated p values are presented in Table 7.1. In a more recent study of 19 patients, 7 of which

Table 7.1 Correlation of the presence and/or severity of symptoms of FXTAS with *FMR1* CGG repeat length and mRNA levels among men

Phenotype	No. of men	CCG repeat range	Correlations	Notes	References
Motor symptoms					
Age of onset—tremor	93 PM	55–200	Repeat: −0.36 ($p=0.001$) mRNA: −0.21 ($p=0.11$)	Spearman's rho, not adjusted by age	Tassone et al. (2007)
Age of onset—ataxia	93 PM	55–200	Repeat: −0.34 ($p=0.002$) mRNA: −0.20 ($p=0.14$)	Spearman's rho, not adjusted by age	
Age of onset—either	93 PM	55–200	Repeat: −0.44 ($p<0.0001$) mRNA: −0.26 ($p=0.03$)	Spearman's rho, not adjusted by age	
FXTAS RS: total motor	54 PM/51 NC/4 IC	Full range	Repeat: 0.27 ($p<0.001$) mRNA: no association	Regression, adjusted for age	Leehey et al. (2008)
FXTAS RS: tremor	54 PM/51 NC/4 IC	Full range	Repeat: 0.11 ($p<0.001$) mRNA: no association	Regression, adjusted for age	
FXTAS RS: ataxia	54 PM/51 NC/4 IC	Full range	Repeat: 0.11 ($p<0.001$) mRNA: no association	Regression, adjusted for age	
FXTAS RS: parkinsonism	54 PM/51 NC/4 IC	Full range	Repeat: 0.06 ($p=0.016$) mRNA: no association	Regression, adjusted for age	
CATSYS: intention tremor	16 PM/2 NC	25–130	Repeat: 0.37 ($p<0.0001$)	Includes only those men who were positive for intention tremor; adjusted for age	Allen et al. (2008)
Postural sway	25 PM (7 with FXTAS)	72–134	Repeat: 0.30 ($p=0.012$)	Regression analysis, adjusted for age	Birch et al. (2014)
ADL frequency change	42 PM with FXTAS	67–142	Repeat: $p<0.05$	Regression analysis	Brega et al. (2009)
IADL limitation	42 PM with FXTAS	67–142	Repeat: $p<0.01$	Regression analysis	
IADL frequency change	42 PM with FXTAS	67–142	Repeat: $p<0.001$	Regression analysis	

Neuropathy					
Neuropathy RS: total	49 PM/46 NC	PM: 60–147	Repeat: $p=0.0005$, all men $p=0.0037$, PM only	Regression, adjusted for age	Berry-Kravis et al. (2007)
Neuropathy RS: reflex	49 PM/46 NC	PM: 60–147	Repeat: $p=0.00007$, all men $p=0.00002$, PM only	Regression, adjusted for age	
Neuropathy RS: vibration	49 PM/46 NC	PM: 60–147	Repeat: $p=0.063$, all men $p=0.42$, PM only	Regression, adjusted for age	
Nerve conduction: tibial conduction velocity	27 PM	55–200	Repeat: −0.42, $p=0.04$ mRNA: −0.52, $p<0.01$	Partial correlation, adjusted for age	Soontarapornchai et al. (2008)
Cognition					
FSIQ	41 PM	55–200	Repeat: −0.32, $p<0.05$ mRNA: −0.05, ns FMRP: 0.25, ns	Pearson correlation; however, in a multiple regression with FSIQ as outcome, no significant association was found	Cohen et al. (2006) and Hessl et al. (2005)
FSIQ	36 PM	50–163	Repeat: −0.54, $p=0.001$ mRNA: ns	Spearman's rho, adjusted by age	
VIQ	36 PM	50–163	Repeat: −0.49, $p=0.004$ mRNA: ns	Spearman's rho, adjusted by age	Cohen et al. (2006)
PIQ	36 PM	50–163	Repeat: −0.48, $p=0.004$ mRNA: ns	Spearman's rho, adjusted by age	
VIQ	33 PM/27 NC	18–130	Repeat: −12.8, $p<0.01$	Regression, adjusted for age and education	Grigsby et al. (2007)
PIQ	33 PM/27 NC	18–130	Repeat: −19.8, $p<0.001$	Regression, adjusted for age and education	

(continued)

Table 7.1 (continued)

Phenotype	No. of men	CCG repeat range	Correlations	Notes	References
VIQ	89 PM/18 NC	20–180	Repeat standardized beta: −0.32 (p=0.002)	Regression, adjusted for age and education	Allen et al. (2011)
PIQ	89 PM/18 NC	20–180	Repeat standardized beta: −0.31 (p=0.002)	Regression, adjusted for age, education, and score on grooved pegboard	
Verbal cognitive decline	89 PM/18 NC	20–180	Repeat standardized beta: 0.29 (p=0.002)	Regression, adjusted for age and education	
Performance cognitive decline	89 PM/18 NC	20–180	Repeat standardized beta: 0.30 (p=0.002)	Regression, adjusted for age, education, and score on grooved pegboard	
Perceptual organization	33 PM/27 NC	18–130	Repeat: −16.1, p<0.001	Regression, adjusted for age and education	Grigsby et al. (2007)
Processing speed	33 PM/27 NC	18–130	Repeat: −20.7, p<0.001	Regression, adjusted for age and education	
Executive function: Behavioral Dyscontrol Scale	33 PM/27 NC	18–130	Repeat: −4.7, p<0.001	Regression, adjusted for age and education	
Executive function: Controlled Oral Word Association Test	33 PM/27 NC	18–130	Repeat: −12.5, p<0.01	Regression, adjusted for age and education	
Executive function: Symbol Digit Modalities Test	33 PM/27 NC	18–130	Repeat: −12.9, p<0.01	Regression, adjusted for age and education	
Executive function: inhibitory control	40 PM	55–200	Repeat: −0.49, p<0.001	Regression analysis; adjusted by age	Cornish et al. (2008)
Cognitive impairment: Mattis Dementia Rating Scale	35 PM/39 NC	Range not reported	Repeat: −0.12; p<0.002	Regression analysis; adjusted for age and education	Sevin et al. (2009)
Executive function: WCST % perseverative responses	35 PM/39 NC	Range not reported	Repeat: 0.3145; p=0.0008	Regression analysis, adjusted for age and education	

Central executive measures	40 PM (6 with FXTAS)/67 NC	PM:55–161/ NC not reported	Repeat: $p<0.001$	Regression analysis	Cornish et al. (2009)
Visual spatial memory	40 PM (6 with FXTAS)/67 NC	PM:55–161/ NC not reported	Repeat: $p<0.001$	Regression analysis	
Psychiatric symptoms					
Somatization	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.38, $p<0.01$ FMRP: ns	Pearson correlation, adjusted for multiple testing	Hessl et al. (2005)
Obsessive-compulsive	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.47, $p<0.001$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Interpersonal sensitivity	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.38, $p<0.01$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Depression	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.44, $p<0.001$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Anxiety	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.41, $p<0.01$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Hostility	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.42, $p<0.01$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Phobic anxiety	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: ns FMRP: ns	Pearson correlation, adjusted for multiple testing	

(continued)

Table 7.1 (continued)

Phenotype	No. of men	CCG repeat range	Correlations	Notes	References
Paranoid ideation	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: 0.39, $p<0.01$ mRNA: 0.45, $p<0.001$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Psychoticism	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.50, $p<0.001$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Global severity index	Repeat: $n=67$ mRNA: $n=51$ FMRP: $n=54$	55–200	Repeat: ns mRNA: 0.45, $p<0.001$ FMRP: ns	Pearson correlation, adjusted for multiple testing	
Major depressive disorder onset age	Repeat: $n=35$ mRNA: $n=35$	58–190	Repeat: ns mRNA: $p=0.015$	Pearson correlation, adjusted for multiple testing	Seritan et al. (2013)
Radiology					
Voxel density of cerebellum, amygdala–hippocampal complex, and thalamus	20 PM (FXTAS status unknown)	55–137	Repeat: $p<0.01$ mRNA: ns FMRP: $p<0.01$	Regression analysis	Moore et al. (2004a)
Whole brain volume	8 PM	56–85	Repeat: $p=0.02$, 2-side test	Regression analysis, not adjusted by age	Cohen et al. (2006) and Loesch et al. (2005b)
Whole brain volume	36 PM (25 with FXTAS)	55–163	Repeat: −0.35, $p=0.04$	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	
Cerebral volume	36 PM (25 with FXTAS)	55–163	Repeat: −0.30, $p=0.08$	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	
Cerebral volume	8 PM	56–85	Repeat: $p=0.02$, 2-side test	Regression analysis, not adjusted by age	Loesch et al. (2005b)
Cerebellar volume	36 PM (25 with FXTAS)	55–163	Repeat: −0.50, $p=0.002$ ($p=0.02$*)	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	Adams et al. (2007) and Cohen et al. (2006)

Cerebellar volume	25 PM (7 with FXTAS)	72–134	Repeat: −0.29 ($p=0.004$)	Regression analysis; adjusted for age	Birch et al. (2014)
Hippocampal volume	36 PM (25 with FXTAS)	55–163	Repeat: −0.32, $p=0.10$ mRNA: $p=0.0035$*	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	Adams et al. (2007) and Cohen et al. (2006)
Ventricular volume	36 PM (25 with FXTAS)	55–163	Repeat: 0.64, $p<0.001$ ($p=0.0047$*)	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	
Whole brain WMI	36 PM (25 with FXTAS)	55–163	Repeat: 0.48, $p=0.004$	Spearman's rho, adjusted by age; multiple testing significance, $p<0.02$	Cohen et al. (2006)
Pathology					
Percentage of neurons with inclusions	11 PM	65–113	Repeat: cortical gray matter: 0.874, $p=0.005$ CA1 pyramidal neurons in hippocampus: 0.929, $p=0.003$ Hippocampal granule cells: 0.964, $p<0.001$	Spearman's rho, adjusted for multiple testing	Greco et al. (2006)
Percentage of astrocytes with inclusions	11 PM	65–113	Repeat: cortical gray matter: 0.886, $p=0.003$ CA1 subregion of the hippocampus: 0.893, $p=0.007$ Cortical white matter: 0.814, $p=0.01$	Spearman's rho, adjusted for multiple testing	

PM premutation, *NC* noncarrier, *IC* intermediate carrier, *RS* rating scale, *WHI* white matter intensities, *ns* non significant

*The sample of Cohen et al. (2006) was a subset of that in Adams et al. (61 PM, 36 with symptoms of FXTAS). However, Adams et al. did not publish correlation coefficients. Thus, we used the results of Cohen et al. in this table, but added *p* values from the regression analyses which included age, mRNA levels, and CGG repeat length

were female, Fraint et al. (2014) did not find an association of the FXTAS rating scale with CGG repeat size. As noted above, Juncos et al. (2011) did not find an association with CGG repeat size and the FXTAS rating scale.

Similarly, using the neuropathy screening scale, Berry-Kravis et al. (2007) found a significant correlation between CGG repeat length and total neuropathy score ($p=0.0005$) and reflex score ($p=0.00007$) after adjusting for age at examination. The correlation obtained from analysis of both carriers and noncarriers indicated that symptoms increased in severity with increasing CGG repeat length (Table 7.1). When premutation carriers were analyzed separately and after adjustment for age, there was a significant correlation of repeat size with the total score ($p=0.0037$); however, this was primarily driven by the reflex score ($p=0.00002$). The vibration score did not correlate with repeat size ($p=0.42$).

In the two studies that used the CATSYS system to evaluate motor symptoms, both found an association of tremor and ataxia with premutation carrier status, but neither study correlated the presence/absence or the severity of symptoms with CGG repeat length (Aguilar et al. 2008; Allen et al. 2008). The one exception was with the follow-up examination of intention tremor in the study of Allen et al. Although there was no significant difference in the presence of intention tremor among noncarriers and carriers, a follow-up analysis found a significant association of tremor intensity and CGG repeat length among those scoring >1 standard deviation above the normative mean for intention tremor (Fig. 7.4) (Allen et al. 2008). This preliminary finding suggests that further studies to evaluate correlation of the severity of motor symptoms with repeat size may help define premutation alleles "at risk."

Postural sway was also found to be associated with CGG repeat size using a swaymeter in a population from New South Wales. They were also able to examine

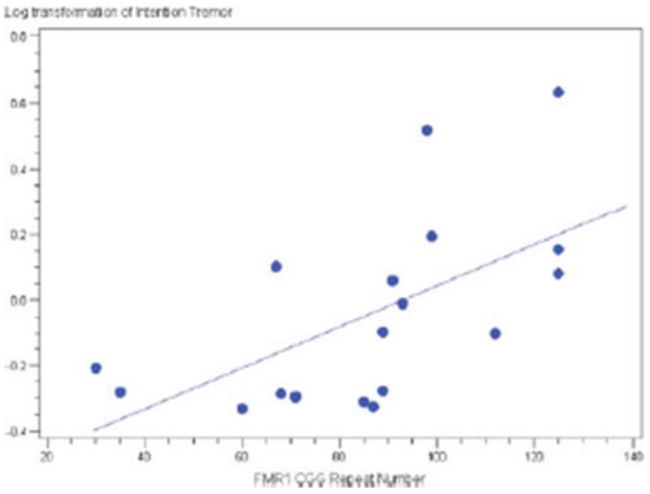

Fig. 7.4 Association of intention tremor intensity (log transformed) as measured by the CATSYS system and repeat length among men who were positive for intention tremor (>1 standard deviation above the mean of a normative population). Taken from Allen et al. (2008)

cerebellar volume in this study and, when included in the regression model, found that the deficit on sway was statistically explained by an effect of decreased cerebellar volume (Birch et al. 2015). That is, they found evidence that cerebellar volume may be a mediator of the effect of CGG repeat size on postural sway.

Last, evaluation of nerve conduction also shows the potential of using such measures to help define "at-risk" alleles as well as potential "prodromal" symptoms of FXTAS (Soontarapornchai et al. 2008). For example, there was a significant correlation between the number of CGG repeats and the mRNA levels with the slowing of tibial nerve conduction velocity among 27 men with the premutation (combined premutation groups with and without FXTAS) (Table 7.1).

Olfactory dysfunction was assessed using the University of Pennsylvania Smell Identification Test (UPSIT) in 41 premutation carriers compared to 42 controls; however, correlation with repeat size was not found (Juncos et al. 2012).

Functional status has also been evaluated. Brega et al. (2009) evaluated 42 men with FXTAS, and found an association with repeat size and a frequency change in Activities of Daily Living (ADLs), the limitations from Instrumental ADLs (IADLs), and a frequency change in IADLs (Table 7.1).

Cognitive Phenotype

Cognitive decline, including memory loss and executive functioning deficits, was described among the initial five cases of FXTAS reported by Hagerman et al. (2001). Since then, cognitive deficits associated with FXTAS have been further characterized, and general findings include a progressive impairment in cognition with significant impairment in executive cognitive functioning (Adams et al. 2007; Brega et al. 2008; Cohen et al. 2006; Grigsby et al. 2006, 2007, 2008; Hessl et al. 2005; Jacquemont et al. 2003; Sevin et al. 2009). See Chap. 3 for more details.

General Intelligence

General intelligence measures have been confirmed to be lower, on average, among men with FXTAS compared with controls (Cohen et al. 2006; Grigsby et al. 2007; Hessl et al. 2005; Jacquemont et al. 2003). In an early report of 11 men with FXTAS individuals who underwent neuropsychological testing, Jacquemont et al. (2003) reported that individuals who had been affected with FXTAS symptoms for the longest period showed lower IQ scores. Similarly, among 36 men with FXTAS, Adams et al. (2007) found an association between a more advanced FXTAS stage (see description above) and decreased performance intelligence quotient (PIQ) and verbal IQ (VIQ).

Executive Function

Executive dysfunction among men with the premutation has been documented in many studies and is outlined in detail in Chap. 3 (Bourgeois et al. 2007; Brega et al. 2008; Cornish et al. 2008; Grigsby et al. 2007, 2008; Jacquemont et al. 2003; Loesch et al. 2005a; Moore et al. 2004a).

In a large cross-sectional sample of men with the premutation, with and without FXTAS, Cornish et al. (2008) examined age-related cognitive deficits. They found that men with the premutation showed statistically significant deficits in their ability to inhibit prepotent responses. This deficit was different from men who did not carry the premutation from age 30 onward. Thus, with increasing age, carriers and noncarriers followed a different trajectory: premutation carriers developed progressively more severe problems in inhibitory control compared to noncarriers. This deficit had a strong co-occurrence among men displaying FXTAS symptoms. Selective attention was also significantly different among carriers and noncarriers, but it was not associated with age. No other cognitive deficits were observed. The authors concluded that inhibitory deficits may impact men with the premutation across their life span and may be a precursor for development of cognitive impairment observed among those with FXTAS.

Brega et al. (2008) asked if the observed executive dysfunction among individuals with FXTAS may secondarily lead to impairment of the other nonexecutive function cognitive skills that have been noted among men with FXTAS. Specifically, they used the results from their previous study of cognition in FXTAS (Grigsby et al. 2007) and assessed the degree to which executive function mediates the deficits in general mental status, verbal and nonverbal intelligence, remote memory, declarative memory, information processing speed, visuospatial functioning, and temporal sequencing. First, their results confirmed that participants with FXTAS had significant executive cognitive dysfunction compared with healthy controls. Hallmarks of the executive dysfunction include deficits in behavioral self-regulation, including disinhibition, working memory, and control of attention. Second, their results suggest that the executive dysfunction significantly influences performance on a range of neuropsychological measures including nonexecutive cognitive function (for further details, see Chap. 3).

Genotype/Phenotype Correlations with Cognitive Measures

To date, a number of studies have indicated a significant association between CGG repeat length and neuropsychological measures among men with the premutation (Table 7.1). Hessl et al. (2005) were first to examine the association between three *FMR1* genetic measures, including CGG repeat length, *FMR1* mRNA level and FMRP level, and full scale IQ (FSIQ) among premutation males ($n=41$). Only CGG repeat length was found to be significantly inversely correlated with FSIQ

using Pearson correlation. However, using multiple regression analysis with FSIQ as an outcome measure and controlling for FXTAS status, none of the *FMR1* measures were significant predictors. This finding contrasts to the association found with psychiatric measures (see below). In a subsequent study, Cohen et al. (2006) assessed the cognitive status a sample of 36 men with the premutation who also underwent radiological studies (11 and 25 without and with signs of FXTAS, respectively). They found that higher CGG repeat lengths were associated with lower FSIQ, performance IQ (PIQ), and verbal IQ (VIQ) after adjusting for age (Table 7.1). Both the younger and the older age groups of carriers showed similar patterns. *FMR1* mRNA was not significantly associated with any of the IQ measures.

In a sample of 60 males (27 controls and 33 premutation carriers with FXTAS), Grigsby et al. (2007) showed that repeat size correlated negatively with measures from the Wechsler Adult Intelligence Scale—Third Edition (verbal IQ, performance IQ, verbal comprehension, perceptual organization, and processing speed). They also identified a significant negative relationship between repeat size and three measures of executive function (Behavioral Dyscontrol Scale, Controlled Oral Word Association Test, and Symbol Digit Modalities Test) (Table 7.1). In an additional study with 89 premutation males and 18 of their noncarrier brothers, VIQ, PIQ, verbal cognitive decline, and performance cognitive decline were all associated with CGG repeat size (Allen et al. 2011) (Table 7.1). Cognitive decline was calculated using the difference of the current IQ measure from the WAIS-III and the premorbid IQ that was determined using the North American Adult Reading Test (NAART).

Cornish et al. (2008) further investigated their finding of a significant progressive inhibitory control among men with the premutation by examining the association with CGG repeat length. Among carriers only, they found that repeat length was significantly correlated with severity of inhibitory control deficit, confirming the findings of others (Table 7.1). A later study of 40 premutation males and 67 noncarrier males revealed an association with CGG repeat size with both central executive function and visual spatial memory. Both measures were defined through a principal components analysis. The central executive function included these measures: spatial span forward task, digit span backward task, and letter number sequencing. The visual spatial memory measure was comprised of spatial span forward task and the dot test (Cornish et al. 2009).

Last, in an independent sample collected by Sevin et al. (2009), a linear relationship was seen between CGG repeat length and the Mattis Dementia Rating Scale (MDRS), a measure of cognitive impairment, after adjusting for age and education (Table 7.1). The study consisted of 35 premutation carrier males over age 50 and 39 intrafamilial controls. When the premutation carriers were divided into two groups (small premutation = 55–69 repeats and midsize/large premutation ≥70 repeats), the authors found that it was the midsize/large premutation group that was at a sixfold increased risk of showing marked cognitive impairment. An association between repeat size and one measure of executive functioning (WCST% perseverative responses) was also identified in this population after adjustments were made for age and education (Table 7.1).

Psychiatric Phenotype

For many neurological disorders, psychiatric symptoms may co-occur and FXTAS does not seem to be an exception. Hessl et al. (2005) used a self-report inventory of current psychological symptoms (SCL-90-R) among 26 and 42 men with the premutation who did and did not have symptoms of FXTAS, respectively. Among those with FXTAS, they found increased symptoms of hostility and paranoid ideation. Among premutation carriers without FXTAS, significant increase in the rates of obsessive-compulsive symptoms and overall symptom severity were identified.

The Neuropsychiatric Inventory (NPI) has also been used to test for psychiatric symptoms among FXTAS individuals. Bacalman et al. (2006) administered the NPI on 14 premutation carriers (CGG repeat number ranging from 62 to 130) with FXTAS compared to 14 age- and education-matched controls. Compared to controls, men with FXTAS had significantly increased total NPI scores, agitation/aggression scores, depression scores, apathy scores, disinhibition scores, and irritability scores. The authors concluded that the neuropsychiatric manifestations of FXTAS appear to cluster as fronto-subcortical dementia.

In a study of 15 FXTAS individuals given a full neurology, psychiatry, and neuropsychology examination, six individuals were given a diagnosis of mood disorder and four individuals were given a diagnosis of anxiety disorder (Bourgeois et al. 2007).

A study of 32 premutation men found that among subjects who reported major depressive disorder, the premutation carriers had a significantly later age of onset compared to the general population (52 vs. 32 years, respectively) (Seritan et al. 2013). This was assessed using the Structured Clinical Interview for DSM-IV-TR (SCID).

Genotype/Phenotype Correlations with Psychiatric Outcomes

The study of Hessl et al. examined three molecular outcomes of the *FMR1* premutation with respect to their influence on psychiatric outcomes among men with the premutation with and without FXTAS symptoms (Hessl et al. 2005). After adjusting for multiple tests, they found that *FMR1* mRNA levels were significantly correlated with all scales of the SCL-90-R except phobic anxiety (Table 7.1): higher levels of *FMR1* mRNA were associated with more severe symptoms. They conducted a post hoc analysis to determine if these correlations were more pronounced among men with FXTAS symptoms compared with those with no symptoms. Interestingly they found that the associations were stronger among those without symptoms. For example, the correlation between the Global Severity Index and the mRNA level was $r=0.70$ ($p=0.001$, $n=18$) among men without symptoms compared with $r=0.35$ ($p=0.04$, $n=36$) among those with symptoms. CGG repeat length was not a significant predictor of any of the scales, except for paranoid ideation ($r=0.39$, $p<0.01$). FMRP levels measured as the percentage of FMRP-positive lymphocytes were not significantly associated with any psychological symptom scale. Finally,

the authors conducted a full analysis of all *FMR1* measures and found that the *FMR1* mRNA level was significantly associated with Global Severity Index ($p=0.01$), independent of repeat length and protein level. Another study found that *FMR1* mRNA was also correlated with major depressive disorder (MDD) age of onset (Seritan et al. 2013) (Table 7.1).

Radiological Phenotype

Prior to the identification of FXTAS, Jakala et al. (1997) reported that men with the premutation may have signs of abnormal brain morphology based on radiological imaging. They found that right and left hippocampal raw volumes averaged over nine premutation males were smaller than those of their age- and sex-matched controls. When normalized for brain area or for coronal intracranial area, premutation males had smaller left hippocampal volumes than controls. Interestingly, no differences in hippocampal measures were found between premutation and full mutation carriers ($n=7$). Subsequently, once FXTAS was established as a premutation-associated disorder, investigators were further motivated to assess men with and without symptoms of FXTAS using neuroimaging. A full description of radiological findings and their association with other FXTAS symptoms are detailed in Chap. 4. Using quantitative MRI on human brain, Moore and colleagues (Moore et al. 2004b) observed that premutation carriers compared to age-matched controls had significantly less voxel density in several brain regions, including cerebellum, amygdala–hippocampal complex, and thalamus. They showed that decreasing voxel density of regions previously identified as decreased relative to controls was dependent of the CGG repeat number such that higher number corresponded to a lower voxel density, consistent with observations of a subgroup of older males with the premutation who present with cognitive decline.

The largest group of premutation men studied using magnetic resonance imaging (MRI) have been presented in a series of studies of subjects evaluated at UC Davis and in University of Colorado (100 males: 36 with FXTAS, 25 asymptomatic premutation carriers, and 39 controls) (Adams et al. 2007; Brunberg et al. 2002; Cohen et al. 2006). Men with FXTAS show brain atrophy and often have subcortical and periventricular white disease. The latter includes white matter disease in the middle cerebellar peduncles (MCP). This sign has become part of the diagnostic criteria for FXTAS due to its high prevalence among those with FXTAS (about 60 %) and low prevalence among those without types of movement disorders (Jacquemont et al. 2003). With respect to brain volumes, these studies have found significantly smaller whole brain and cerebellar volumes and larger ventricular volume in affected carriers when compared with both unaffected carriers and controls. No differences in hippocampal volume were found between those with FXTAS and either the unaffected carriers or the controls. Furthermore, unaffected carriers did not differ from controls on any measured volumetric parameters. The authors of these studies conclude that the fact that unaffected and affected groups of men with the premutation differed significantly on many MRI measures, combined with the finding that unaffected men

and controls did not differ, suggests that the radiological changes are due to the presence of FXTAS; they are not due to a direct effect of the premutation.

The work of Loesch et al. (2005b, 2008) confirms many of the above findings, but there are some notable differences. Although their sample size was considerably smaller (eight older premutation males not selected for symptoms of FXTAS and 21 age-matched controls), they found that premutation carriers showed significantly increased total hippocampal volume. They found that premutation carriers had significantly reduced volumes for whole brain, cerebrum, cerebellum, and cerebral cortex (Loesch et al. 2005b). Interestingly, they studied two younger male carriers, ages 52 and 39 years, who did not show neurological symptoms, but did have the characteristic MCP sign (Loesch et al. 2008). Thus, they proposed that the MRI signs may be an early indicator of FXTAS. Clearly, more research is needed to determine the progression of FXTAS neuroimaging findings.

Genotype/Phenotype Correlations with Radiological Outcomes

As with the other symptoms of FXTAS, the radiological findings appear to increase in magnitude with increasing repeat length. Although there are methodological differences in measurements and analyses, reports suggest that CGG repeat length was associated with volume or signal intensity in many brain regions among men with the premutation including reduced whole brain volume (Fig. 7.5) (Cohen et al.

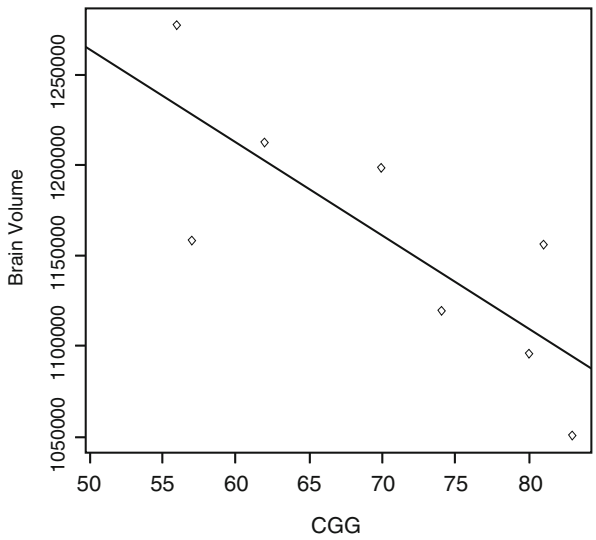

Fig. 7.5 Scatterplot of the measures of brain volume (in cubic millimeter) against the number of CGG repeats for eight patients. Fitted regression line: Brain volume = 1,523,477 + 5183 × CGG. Taken from Loesch et al. (2005b)

2006; Loesch et al. 2005b), reduced cerebral volume (Cohen et al. 2006; Loesch et al. 2005b), reduced cerebellar volume and increased ventricular volume (Adams et al. 2007; Cohen et al. 2006), and increased whole brain white matter hyperintensity (Cohen et al. 2006) (Table 7.1). Reduced cerebellar volume was confirmed in a more recent study (Birch et al. 2015) (Table 7.1). Volumes of brain stem, hippocampus, and cerebellar white matter hyperintensity did not show association with CGG repeat length (Cohen et al. 2006). Interestingly, Adams et al. (2007) found that elevated *FMR1* transcript level, not CGG repeat length, was associated with reduced hippocampal volume (Table 7.1). Several of these associations were not statistically significant once adjusted for multiple tests; however, the pattern is the same among different reports. As expected, the severity of some of the radiological signs (reduced cerebellar volume, reduced hippocampal volume, and increased ventricular volume) was associated with a more advanced clinical stage among men with FXTAS (Adams et al. 2007).

Pathology Phenotype

In 2002, Greco et al. (2002) reported the postmortem results on four men with FXTAS. Each of the four individuals showed intranuclear inclusions in neurons and astrocytes. From two of the brains, they found that the inclusions were located throughout the cerebrum and brain stem and were most numerous in the hippocampal formation. In the cerebellum, they found dropout of Purkinje cells, Purkinje axonal torpedoes, and Bergmann gliosis. They did not find inclusions in the Purkinje cells, but inclusions were found in a few neurons in the dentate nucleus and diffusely in cerebellar astrocytes.

This report was followed up in 2006 with an additional seven men. Based on 11 men who had FXTAS, Greco et al. (2006) identified the three most prominent neuropathological characteristics: (1) significant cerebral and cerebellar white matter disease, (2) associated astrocytic pathology with dramatically enlarged inclusion-bearing astrocytes prominent in cerebral white matter, and (3) the presence of intranuclear inclusions in both brain and spinal cord. For detailed description, see Chap. 5.

Genotype/Phenotype Correlations with Pathology Outcomes

Greco et al. (2006) emphasized that the most striking finding among the 11 cases of men with FXTAS was the highly significant association between the number of CGG repeats and the numbers of intranuclear inclusions in both neurons and astrocytes (Table 7.1, Fig. 7.6). This suggests that CGG repeat length is an important predictor of brain involvement in men with the premutation. Importantly, they also found an association with age of death: there was a significant decrease in the age of death with increasing CGG repeat length ($n = 10$; rho = –0.896; $p < 0.001$), which remained significant after adjustment for multiple testing.

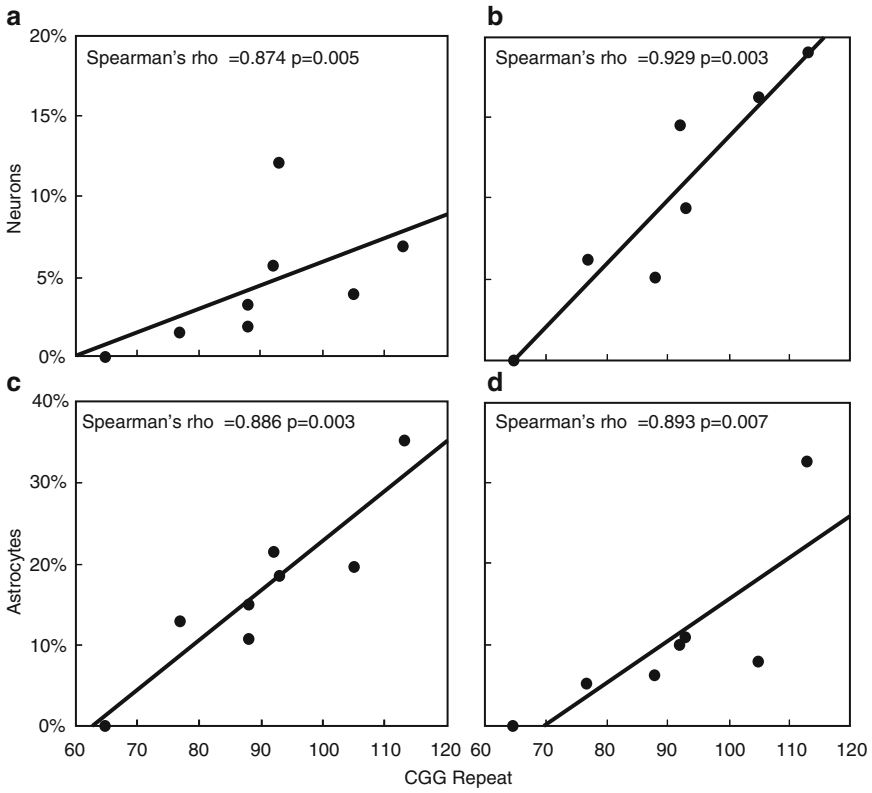

Fig. 7.6 Scatterplots demonstrating significant correlations between CGG trinucleotide repeat number and percentage of neurons and astrocytes with intranuclear inclusions. Frontal cortical gray matter neurons (**a**) and astrocytes (**c**). Hippocampal neurons (**b**) and astrocytes (**d**) of the CAI pyramidal region. Spearman's rho values and *p* values are given for each plot. Taken from Greco et al. (2006)

Genotype/Phenotype Correlations Among Women with the Premutation

Women who carry the premutation are more rarely affected with FXTAS compared with men. Although some women have the full spectrum of symptoms of FXTAS (Biancalana et al. 2005; Hagerman et al. 2004; Zuhlke et al. 2004), others have a more mild presentation (Berry-Kravis et al. 2003; Jacquemont et al. 2004). This is presumably due to the location of the *FMR1* gene on the X-chromosome and the consequent random X-chromosome inactivation. Not only is FXTAS less penetrant, but it may also be less severe and/or present with different symptoms. The overall clinical manifestations of FXTAS among women are reviewed in the previous chapters. Here we will examine the few studies that have begun to correlate FMR1 molecular outcomes with symptoms of FXTAS with the caveat that most studies

have not found a difference among female premutation carriers and controls for many of the symptoms of FXTAS due to the lower frequency of FXTAS among women with the premutation. In addition, we emphasize that the correlation between CGG repeat length and mRNA levels in females is not as strong as in males, again, presumably due to X-chromosome inactivation. For example, in Allen et al. (2004), the CGG repeat length among individuals with a wide range of repeat lengths explained about 40 % of the variation in mRNA levels in males whereas it only explained 6 % in females. When X-chromosome inactivation was included in the model, the correlation only increased to 9 %. Similarly, in the study of Hessl et al. (2005), the correlation between CGG repeat size and mRNA levels was 0.53 ($p<0.0001$) among males with the premutation and only 0.29 ($p<0.01$) among females with the premutation.

Clinical Neurological Phenotype

Studies have used many of the same tools to measure symptoms of FXTAS as those used for men with the premutation, including the FXTAS rating scale and the neuropathy screening scale. Initially, no differences in mean scores between carriers ($n=14$) and controls ($n=7$) were identified with the FXTAS rating scale in a blinded videotape study (Berry-Kravis et al. 2003). Similarly, in a larger study comparing 82 women with the premutation and 39 controls, no differences in the total or domain subscores of the FXTAS rating scale were detected. However, when an adjustment was made for X-chromosome activation ratio, a significant association between CGG repeat length and the ataxia subscore was seen in a model adjusted for age ($p=0.03$) (Leehey et al. 2008). No association was observed with *FMR1* mRNA levels as measured in leukocytes.

Neuropathy screening scale scores were evaluated for 73 women with the premutation and 32 women without the premutation (Berry-Kravis et al. 2007). Using both carriers and noncarriers in the analysis and adjusting for age, there was a significant correlation between repeat length and total neuropathy score ($p=0.04$) and trends for vibration ($p=0.12$) and reflex ($p=0.08$) scores. However, it is not clear if their significant correlations were driven primarily between noncarriers and carriers or if there was an increase in severity of symptoms with repeat lengths within the premutation range. Irrespective, the pattern is similar to that found in men with the premutation. The X-chromosome activation ratio was not measured in this study.

A large study of 146 premutation carrier females and 69 age-matched controls was conducted by Coffey et al. (2008) to examine comorbid medical conditions of those with FXTAS. From that study, a subset of 18 women with "definite" or "probable" FXTAS were compared with 39 age-matched controls and were found to have greater medical comorbidity: they showed increased prevalence of thyroid disease, hypertension, seizures, peripheral neuropathy, and fibromyalgia in addition to the significantly increased frequency of tremor and ataxia. There was no difference in the X-chromosome activation ratio between premutation carriers with and without

FXTAS symptoms ($p=0.92$). Although not directly analyzed, mean CGG repeat length did not appear to differ between these groups.

In another study, among female premutation carriers the penetrance of FXPOI, thyroid disease, and chronic muscle pain was found to be 18.6 %, 15.9 %, and 24.4 %, respectively (Rodriguez-Revenga et al. 2009).

Psychiatric Phenotype

The largest study to date on the correlation between the *FMR1* molecular measures and the psychiatric consequences of the premutation was done by Hessl et al. (2005). This study evaluated 144 women with the premutation using the SCL-90. Among women with symptoms of FXTAS ($N=22$), there was a significant increase in the rates of somatization, obsessive-compulsive symptoms, and overall symptom severity. Among those without FXTAS ($N=122$), there was a significant increase in obsessive-compulsive symptoms, and they were significantly below the average on phobic anxiety and paranoid ideation. No significant associations were found with SCL-90 scores and CGG repeat length, mRNA, or protein levels. In a post hoc exploratory analysis, women with an X-activation ratio of less than 0.5 (i.e., more cells with an active premutation) showed a significant correlation between mRNA levels and anxiety ($r=0.57$, $p<0.001$), although in this subgroup no other significant correlations were evident with mRNA, CGG repeat length, or FMRP and any other SCL-90-R symptom.

Radiological Phenotype

Findings to date suggest that the radiological hallmarks observed in men with FXTAS appear to be less severe in women with FXTAS, although the patterns of changes are the same. The largest study to date was conducted by Adams et al. (2007). They compared MRI results from 15 women with FXTAS, 20 unaffected premutation carriers, and 11 controls as well as 36 men with FXTAS, 25 unaffected premutation carriers, and 39 controls. Similar to men, they found reduced brain volumes and involvement of the middle cerebellar peduncles (MCP sign) compared with unaffected carriers and with controls. These differences were statistically significant only among affected women of age 70 years and above. Unlike men, women with FXTAS did not show significant differences in cerebellar or ventricular volume.

This milder phenotype alone reduces the power to detect associations with *FMR1* molecular parameters. When Adams et al. (2007) examined CGG repeat length and mRNA levels among men with the premutation, they found a significant association between CGG repeat length and reduced cerebellar volume and increased ventricular volume (see above). No associations were identified in women. Examination of

mRNA levels indicated an association with reduced hippocampal volume in both men ($p=0.003$) and women ($p=0.04$) with the premutation; however, statistical significance was not reached once adjusted for multiple testing.

Genotype/Phenotype Correlations in Premutation Carriers Without FXTAS

Carriers of the *FMR1* premutation are at risk of developing FXTAS; however, many clinical involvements have been observed in premutation carriers during the entire life span (Hagerman and Hagerman 2013). Currently it is not known which phenotypic features may be present in all premutation carriers or which are specific to FXTAS. In 29 adult women premutation carrier without FXTAS, Goodrich-Hunsacker et al. (2011b) reported a significant association between changes in cognitive functions and CGG repeat number but not with *FMR1* mRNA levels. Interestingly, they also reported that although adult female asymptomatic (non-FXTAS) premutation carriers had a poor performance on cognitively demanding tasks (Goodrich-Hunsaker et al. 2011a, c), they showed enhanced performance on manual and oral motor reaction time as function of the CGG repeat number (Goodrich-Hunsaker et al. 2011c). Association between genetic variability in the *FMR1* gene and executive cognitive functioning has also been documented in male carriers with FXTAS with a poor task performance on response inhibition (Grigsby et al. 2006, 2007; Hocking et al. 2012; Wong et al. 2014) and visuospatial working memory (Hocking et al. 2012) in those carrying an allele with a higher CGG repeat number. Psychological difficulties including obsessive-compulsive and psychotic symptoms were related to the *FMR1* mRNA levels but not to the CGG repeat number regardless their FXTAS status (Hessl et al. 2005). Finally, epigenetic modifications within the *FMR1* gene have been found to affect dysexecutive and psychiatric symptoms including anxiety in women premutation carriers (Cornish et al. 2015).

Although further studies determining the extent of the CGG repeat toxicity, particularly in premutation carriers who are asymptomatic for a late-onset neurodegenerative disorder, are warranted, these findings may help to delineate a neurocognitive endophenotype in those that may be at potential risk for developing a neurodegenerative disorder later in life.

Summary

Based on the evidence gathered to date from many studies, it is clear that CGG repeat length correlates with neurological, cognitive, radiological, and pathological phenotypes associated with FXTAS in men with the premutation (Table 7.1). The correlation with CGG repeat number has also been well documented with executive function deficits in asymptomatic premutation carriers (Farzin et al. 2006; Hessl

et al. 2005; Hunter et al. 2008). With increasing repeat length, the severity of observed symptoms and manifestations increases. To date, the effect appears to be linear, although no direct test has been conducted. The impetus to examine this further is provided by the other premutation-associated disorder, fragile X-associated primary ovarian insufficiency (FXPOI). Several studies have shown a nonlinear relationship with repeat size and risk for FXPOI (Allen et al. 2007; Ennis et al. 2006; Mailick et al. 2014; Tejada et al. 2008). This nonlinear pattern may provide insight to the molecular mechanism underlying the influence of repeat length on outcome for both disorders. Some studies of FXTAS have also identified an association of *FMR1* mRNA levels and FXTAS symptoms (Table 7.1); however, these associations are not as frequent or as strong as those of repeat length. The considerable variability in mRNA measurements may reduce the power to detect associations. Furthermore, there may be significant variability from tissue to tissue; thus, measurements conducted on leukocytes may be only weakly correlated with those levels in the target tissues associated with FXTAS. Specifically, *FMR1* mRNA expression levels appear to be different in different regions of the human brain and also higher than the levels observed in peripheral blood leukocytes (Tassone et al. 2004). On the contrary, the CGG repeat number appears to be similar in different tissues as suggested by postmortem studies and size comparison in different tissues. The problem of variability in measurement becomes exacerbated in women with the premutation due to X-chromosome inactivation. This is true with respect to symptomatology of FXTAS as well as *FMR1* molecular attributes (e.g., mRNA and FMRP levels). Thus, associations are weak if present at all.

Importantly, more work is needed to better define "high-risk" alleles for FXTAS. To date, premutation alleles are defined based on their instability. More specifically, they are defined as alleles with repeat lengths that can expand to the full mutation in one generation. There is a need to better define the attributes of alleles that lead to a high risk of FXTAS. Repeat lengths will certainly be in this definition, but we need to identify the lower range of premutation repeat lengths that lead to a significant risk of FXTAS. For example, Jacquemont et al. (2006) suggested that alleles with 70 repeats or more lead to a high risk of FXTAS. Additional studies are also needed to understand if the repeat structure (i.e., AGG interspersion pattern) affects this risk in addition to repeat length. Irrespective, the studies reviewed here have made significant progress toward understanding the correlation of *FMR1* genotype measures and phenotype outcomes associated with FXTAS. Further elucidation will be of great significance to understand the basic mechanism of CGG repeat toxicity, to improve genetic counseling, and to improve current diagnostic tools.

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Chapter 8
Mouse Models for FXTAS and the Fragile X Premutation

Molly M. Foote, Milo Careaga, Ronald A.M. Buijsen, Robert F. Berman, Rob Willemsen, and Renate K. Hukema

Abstract The use of model organisms is essential in order to understand the pathogenesis of many types of human disease, and this is particularly true for the study of genetic diseases such as fragile X syndrome and fragile X-associated tremor/ataxia syndrome (FXTAS). In reverse genetics, the functional study of a gene starts with the question of how a possible phenotype may derive from a specific genetic sequence (disease-causing mutation in a gene). As a first step, a gene function is purposefully altered and the effect on the normal development and/or behavior of the model organism is analyzed. In addition to providing knowledge about the cellular and molecular mechanisms underlying specific genes and their functions, animal models of human disease also provide systems for developing and validating therapeutic strategies.

The choice of which animal model is most suitable to mimic a particular disease depends on a range of factors, including anatomical, physiological, and pathological similarity; presence of orthologs of genes of interest; and conservation of basic cell biological and metabolic processes. In this chapter, we will discuss two model organisms, a mammalian vertebrate (mouse) and an invertebrate model (fly), which have been generated to study the pathogenesis of FXTAS and the effects of potential therapeutic interventions. Both mouse and fly models have proven invaluable for the study of the pathophysiology of FXTAS, including insights into the role of mutant mRNA in this disease (i.e., RNA gain-of-function mechanisms, see Chap. 6).

Keywords Mouse • Drosophila • Animal models • Behavior • Early onset • Therapies • RAN translation • microRNA • Intranuclear inclusions • FMRP • FXTAS • Premutation

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Mouse Models for FXTAS

The laboratory mouse (*Mus musculus*) has been preeminent for the study of human genetic disease for decades, from simple Mendelian-inherited disease to complex multigenic diseases. In the case of FXTAS, it is striking that transgenic mouse models expressing expanded CGG repeat tracts were already available long before FXTAS had been discovered. This is because these genetically modified mice with CGG repeat expansions were initially generated to study the timing and mechanisms of repeat instability, rather than to specifically model what we now know as the neurodegenerative disease FXTAS.

Repeat Instability

The first of these transgenic mouse models expressed 81 CGG repeat units in the human *FMR1* promoter, including two interruptions (AGG and CAG), but with a pure $(CGG)_{60}$ tract (Bontekoe et al. 1997). Although in humans, dynamic and often dramatic instability of this repeat length upon transmission to the next generation has been demonstrated, these transgenic mice showed a stable inheritance in all mitosis and meiosis conditions studied. Similar results were obtained in transgenic mice expressing either an interrupted $(CGG)_{86}$ repeat tract or a pure $(CGG)_{97}$ repeat tract in the human *FMR1* promoter (Lavedan et al. 1997, 1998). Several hypotheses were put forward to explain the discrepancy between human and murine instability of the CGG repeat. One postulation was that the AGG and CAG interruptions, known to stabilize the repeat tract, were involved. However, the pure $(CGG)_{97}$ repeat also showed a stable transmission. Alternatively, the genomic localization of the CGG repeat had been proposed as a possible explanation for the observed CGG repeat stability. Finally, other factors such as the presence of flanking sequences were considered to be involved as well. Subsequently, several transgenic lines carrying CGG repeats were generated with the use of yeast artificial chromosomes (YACs), which allow the inclusion of large flanking sequences and other regulatory elements. YAC transgenic mice expressing CGG repeats of varying lengths have been used to investigate the effect of potential *cis*- and *trans*-factors promoting expansions (Peier and Nelson 2002). While length-dependent intergenerational instability was found in these animals, only small expansions and contractions were detected and the dynamic mutations never observed in fragile X syndrome. Apparently, this approach was not sufficient to recapitulate all aspects of CGG repeat instability in humans, possibly due to the random integration of the transgene.

To circumvent this phenomenon, a knock-in mouse model has been generated in which the murine $(CGG)_8$ repeat within the endogenous *Fmr1* gene was replaced by a human $(CGG)_{98}$ repeat using a homologous recombination technique in embryonic stem (ES) cells (Bontekoe et al. 2001). When generating the targeting construct containing the human $(CGG)_{98}$ repeat, minimal changes to the murine *Fmr1* promoter

were made. These $(CGG)_{98}$ knock-in mice showed moderate instability on paternal and maternal transmission with both expansions and contractions observed. Since expansions, albeit moderate in length, were observed these $(CGG)_{98}$ knock-in mice were bred over several generations to establish transgenic lines with larger expanded alleles (>200 CGGs) (Bontekoe et al. 2001). Indeed, repeat instability in these knock-in mice has resulted in up to 230 CGG repeats. A bias toward expansions over contractions was observed, with the largest expansion being 43 CGGs (Brouwer et al. 2007). Although it was expected that these longer CGG repeat expansions would eventually lead to methylation of the *Fmr1* gene, to date, and despite CGG repeat tracts well over 200 CGGs long, no methylation of the promoter region has been detected. Additional studies also demonstrated somatic instability, although the increase was relatively small (less than 10 CGG units) (Willemsen et al. 2003). Another similar knock-in mouse has been reported which had an initial $(CGG)_{118}$ tract (Entezam et al. 2007). These mice also show high repeat instability with a bias toward expansions. In yeast and *Escherichia coli* it has been reported that the rate of instability is changed in different repair-deficient strains (Iyer et al. 2000; Jakupciak and Wells 1999; White et al. 1999). This phenomenon prompted these authors to investigate the effect of ATR (ataxia telangiectasia and rad3-related kinase) deficiency, a potential DNA damage checkpoint protein, on CGG repeat instability in their knock-in mice as well. ATR heterozygosity led to an increased frequency of expansion of alleles, however, only of maternal origin. This suggests that expansion can occur prior to fertilization of the oocyte (Entezam and Usdin 2008). Recently, it was reported that two DNA damage repair pathways are involved in instability of the CGG repeat expansion. Firstly, the transcription coupled repair protein ERCC6/CSB protects against repeat expansions (Zhao and Usdin 2015). Secondly, the base pair excision pathway is involved, since heterozygosity for a hypomorphic Polβ mutation reduces the expansion frequency (Lokanga et al. 2015).

Molecular Findings

The description of a new late-developing neurodegenerative syndrome in elderly males carrying the fragile X premutation, FXTAS, (Hagerman et al. 2001) led to renewed interest in both $(CGG)_{98}$ and $(CGG)_{118}$ knock-in mice (from now on called exCGG mice), because studying exCGG mice as they age could provide important insights into the mechanistic basis of FXTAS. Biochemical analysis of the brains of these mice revealed elevated *Fmr1* mRNA levels and reduced FMRP expression. Importantly, elevated *Fmr1* mRNA levels were detected throughout development from 1 to 72 weeks of age (Willemsen et al. 2003). Since *Fmr1* mRNA levels were already elevated by 1 week of age, the potential exists for developmental consequences of excess transcript production in human premutation carriers, in addition to the neurodegenerative changes of FXTAS. On average, a twofold increase of *Fmr1* mRNA levels (range 1.5- to 3-fold) was reported, but the increase was not correlated to the size of the repeat length in the initial knock-in mouse model

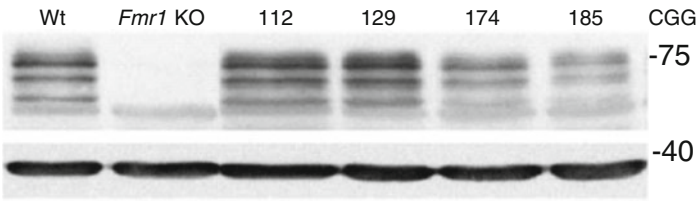

Fig. 8.1 FMRP levels at different (CGG) *n* lengths in 100-week-old whole brain homogenates. FMRP levels decrease mildly (70 %) as (CGG) *n* length increases

(Brouwer et al. 2008b). A more recent study, consisting of 57 wild-type and 97 expanded-CGG-repeat mice carrying up to ~250 CGG repeats, however, found a strong positive correlation between CGG repeat length and *Fmr1* mRNA (Ludwig et al. 2014). This correlation between *FMR1* mRNA levels and repeat size is consistent with observations in human carriers (Allen et al. 2004; Kenneson et al. 2001; Ludwig et al. 2014; Primerano et al. 2002; Tassone et al. 2000), as described in Chap. 6. It should be noted that most human data were obtained using qRT-PCR analysis on RNA isolated from peripheral blood or EBV-transformed lymphoblasts and not brain homogenates. However, analysis of *FMR1* mRNA levels in postmortem brain samples showed similar findings (Ludwig et al. 2014). A study from Tassone et al. (2004) has shown that *FMR1* mRNA levels are higher in brain than in blood. However, the ratio of *FMR1* mRNA levels in premutation carriers appears to be higher in blood (3.8×) than in brain (1.5×). Entezam and colleagues (2007) were able to show a direct relationship between repeat size and *Fmr1* mRNA levels in brains of their exCGG mice; however, the number of mice studied for the different repeat sizes was limited.

Both exCGG mouse strains showed an inverse correlation between CGG repeat length and FMRP expression in brain using semiquantitative Western blot techniques (Brouwer et al. 2008a; Entezam et al. 2007), as shown in Fig. 8.1. The degree of change in FMRP expression in the brain appears to be region specific, with significantly reduced expression throughout the brain and relatively high expression in the hippocampus (Brouwer et al. 2007; Entezam et al. 2007). Thus, in spite of the elevated levels of *Fmr1* transcripts reduced FMRP expression was found. This apparent paradox was explained by a hypothetical model in which the exCGG expansion in the 5′UTR of the transcript hampers the initiation of translation (Primerano et al. 2002). How reduced FMRP levels may contribute to enhanced transcriptional activity is still unknown.

Neuropathology

Further characterization of these mouse models demonstrated the presence of ubiquitin-positive inclusions in many neuronal nuclei of the brain (Entezam et al. 2007; Hashem et al. 2009; Willemsen et al. 2003), a key neuropathological feature

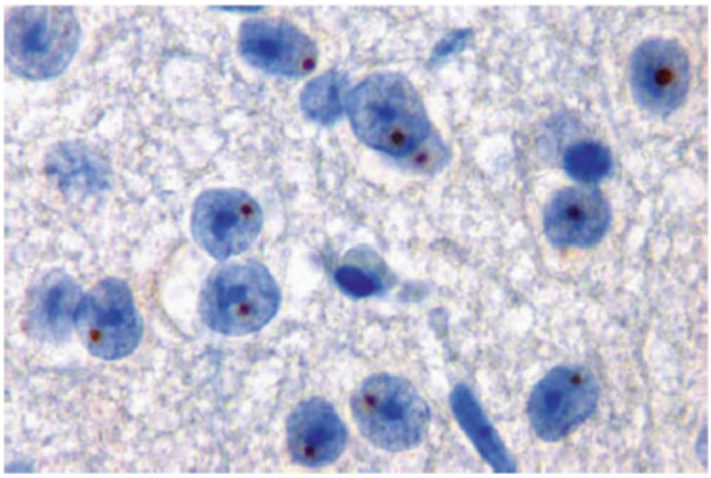

Fig. 8.2 Immunohistochemistry with antibodies against ubiquitin in brain tissue (colliculus inferior) of 100-week-old exCGG mice. Many intranuclear ubiquitin-positive inclusions (*brown* precipitate) are present

of FXTAS in humans (Greco 2002; Greco et al. 2006). A comprehensive study examining the presence of these intranuclear neuronal inclusions in various brain regions at different ages demonstrated the occurrence of intranuclear neuronal inclusions throughout the brain (see Fig. 8.2) with high percentages of inclusions in specific brain structures, including olfactory nucleus, parafascicular thalamic nucleus, medial mammillary nucleus, colliculus inferior, cerebellum, amygdala, cerebral cortex, hippocampus, hypothalamus, and pontine nucleus at 72 weeks of age (Brouwer et al. 2008a, b; Willemsen et al. 2003). Inclusions were also found in astrocytes and Bergmann glia (Wenzel et al. 2010). Furthermore, the average size of the inclusions within one specific brain area correlated significantly with the age of exCGG mice. In younger mice, smaller sized inclusions were found compared to older mice. Interestingly, the gradual increase in the size of the inclusions and the percent of ubiquitin-positive neurons over the lifetime likely parallel the progressive development of the neurological phenotype of FXTAS in humans (Jacquemont et al. 2004). A correlation was seen between the presence of intranuclear inclusions in distinct brain regions and the clinical features in exCGG mice (Willemsen et al. 2003). In an attempt to further characterize the constituents of the inclusions, a systematic analysis was performed to localize a panel of protein candidates (related to FMRP or other disorders with inclusions) using double-labeling immunohistochemistry. In addition to ubiquitin, molecular chaperone Hsp40, 20S proteasome complex, DNA repair-ubiquitin-associated HR23B, SAM-68, and a CGG repeat-associated non-AUG (RAN) translation product, FMRpolyG accumulated in the inclusions (Bergink et al. 2006; Hukema et al. 2015; Sellier et al. 2010, 2013; Todd et al. 2013; Willemsen et al. 2003) (see Chap. 6). As observed for the human intranuclear inclusions, FMRP could not be detected in the inclusions from mouse brain,

although *Fmr1* mRNA was detectable (Sellier et al. 2010). In conclusion, the presence of inclusions in exCGG mice points to a direct role of the *Fmr1* gene, by either CGG expansion in the transcript per se or elevated mRNA levels.

Purkinje cell degeneration, another neuropathological finding in human FXTAS, could only be observed in one of the exCGG mouse lines (Entezam et al. 2007). In a transgenic mouse model using the Purkinje cell specific L7 promoter to express an expanded rCGG repeat outside the context of the *FMR1* gene, intranuclear inclusions were found in Purkinje cells. Ubiquitin, 20S core complex of the proteasome, Hsp40, and Rad23B were consistently present in these inclusions as well. In addition, these mice show axonal swelling indicating cell damage and Purkinje cell dropout (Hashem et al. 2009).

Chen et al. (2010) reported early developmental defects and altered synaptic morphology in hippocampal neurons from heterozygous exCGG female mice. High levels of *Fmr1* mRNA and modest reduction of FMRP levels were observed in these mice, leading to reduced viability of exCGG neurons, consistent with the mRNA toxicity and neurodegeneration associated with FXTAS (Samaraweera et al. 2013).

Immunological Findings

Increased frequencies of autoimmune and autoinflammatory disorders have been reported in female premutation carriers, including a significantly increased risk for developing autoimmune thyroid disorders and Fibromyalgia (Coffey et al. 2008; Winarni et al. 2012). In both human and murine premutation carriers cellular immune responses appear impaired, with decreased productions of cytokines in response to immune challenge in both peripheral blood monocular cells (PBMC) and splenocytes in humans and mice, respectively. Additionally, in human premutation carriers, decreased relative levels of $CD25^+$ T cells have been observed; CD25 is a marker for activated T cells which is also expressed highly on regulatory T cells (Careaga et al. 2014). As impairments in the immune system are associated with an increased risk for developing autoimmunity, the immunophenotype observed in premutation carriers may also be related to the increased frequencies of autoimmune and autoinflammatory disorders previously reported in these individuals (Mackay et al. 2010; Sleasman 1996). In male carriers diagnosed with FXTAS, an increased immune response rather than an impaired one has been observed, which suggest that immunological processes may be involved in the pathology of FXTAS (Marek et al. 2012). Although exactly how the immune system is involved in FXTAS pathology is unknown, studies in *Drosophila* suggest that key immune pathways may be involved. The Toll signaling pathway, which is a key pathway for the immune system, has been found to be essential for RNA toxicity in a *Drosophila* model of another expanded repeat neurodegenerative disease (Samaraweera et al. 2013). This suggests that a similar immune mechanism may be involved in the hypothesized RNA toxicity in FXTAS as well.

Behavioral Phenotype

Several features of human FXTAS have been modeled in exCGG mice including late-onset ataxia, memory impairments, and sensorimotor gating deficits. Motor learning and visuomotor deficits have been reported in the exCGG mice utilizing skilled forelimb reaching tasks (Diep et al. 2012; Von Leden et al. 2014). A significant decrease in motor performance with age was found in exCGG and rCGG mice using an accelerating rotarod apparatus (Hashem et al. 2009; Van Dam et al. 2005). This age-dependent decline in neuromotor performance may be related to the progressive movement and gait disorder in human patients with FXTAS (Hagerman et al. 2001). Additionally, these motor deficits appear to be CGG repeat length dependent, in which high CGG repeat length exCGG mice performed worse during a ladder rung test and a skilled forelimb reaching task compared with lower CGG repeat length exCGG mice (Diep et al. 2012; Hunsaker et al. 2011).

Spatial learning and memory was also impaired in exCGG mice illustrated by poor performance in Morris water maze when tested at 52 weeks of age (Van Dam et al. 2005). Borthwell et al. (2012) showed that the exCGG mice were found to have additional hippocampal-dependent learning and memory deficits based on their impaired performance in temporal order and high interference novelty detection tests. Additionally, an age-dependent impairment of spatial information processing was identified in the exCGG mice using a metric and topological spatial processing task (Hunsaker et al. 2009). Results from these studies parallel the cognitive impairments, including memory problems and executive function deficits, some eventually progressing to dementia, in many patients with FXTAS (Cornish et al. 2008; Grigsby et al. 2008; Hagerman and Hagerman 2004). Interestingly, this deficit in hippocampal-dependent learning and memory is also correlated with CGG repeat length (Borthwell et al. 2012; Hunsaker et al. 2010, 2012). These cognitive defects may also be associated with high concentrations of intranuclear ubiquitin inclusions in the hippocampus of FXTAS patients and modeled in exCGG mice (Greco 2002; Willemsen et al. 2003).

Sensorimotor gating is a neuronal process for filtering out unnecessary stimuli or information in the brain (Braff et al. 2001). Deficits in sensorimotor gating, measured by prepulse inhibition (PPI) of the acoustic startle response, are associated with various neurological disorders, including FXTAS (Schneider et al. 2012). Altered baseline startle responses and an age-dependent PPI deficit have also been found in exCGG mice (Renoux et al. 2014).

Ongoing research is focusing on screening inducible exCGG mice, that express different repeat sizes (90–180 CGGs) in specific cell types in the brain (neurons or astrocytes), using a battery of behavioral assays. The rationale behind this strategy is to define the relative individual contributions of pathology in neurons and astrocytes for full expression of the disease, as well as to establish critical periods for disease and possible reversibility of pathology when expression of the CGG repeat expansion is halted.

Psychopathology

Male premutation carriers may develop a variety of neuropsychological symptoms, including mood and anxiety disorders (Bacalman et al. 2006; Grigsby et al. 2008; Hessl et al. 2005; Hunter et al. 2008; Jacquemont et al. 2004) as discussed in Chap. 3. Thus far knowledge about the origin of the psychopathology is limited, but elevated stress hormone levels have been suggested as a possible explanation. Studies have reported that elevated *FMR1* mRNA levels were associated with increased psychopathology, including anxiety, depression, and irritability in adult premutation carriers, with or without symptoms of FXTAS (Bacalman et al. 2006; Bourgeois et al. 2007; Grigsby et al. 2008; Hessl et al. 2005; Hunter et al. 2008; Jacquemont et al. 2004). Additional support for an elevated stress response and thus aberrant hypothalamic–pituitary–adrenal (HPA) axis function in premutation carriers comes from reports demonstrating ubiquitin-positive intranuclear inclusions in the pituitary gland of autopsy brain material from patients with FXTAS (Greco et al. 2007; Louis et al. 2006). Additionally, fMRI studies have reported reduced amygdala activation in male premutation carriers in response to fearful faces, which may contribute to the etiology of impaired social cognition in these patients as well (Hessl et al. 2007). Similarly, studies in exCGG mice at 72 weeks of age point to an increased anxiety phenotype in the open-field behavior test (Van Dam et al. 2005). Further characterization of the HPA axis physiology in exCGG mice revealed dramatically elevated corticosterone levels in serum in response to a mild stressor, as well as intranuclear ubiquitin-positive inclusions in both the pituitary and the adrenal glands of 100 weeks old mice (Brouwer et al. 2008b) (see Fig. 8.3).

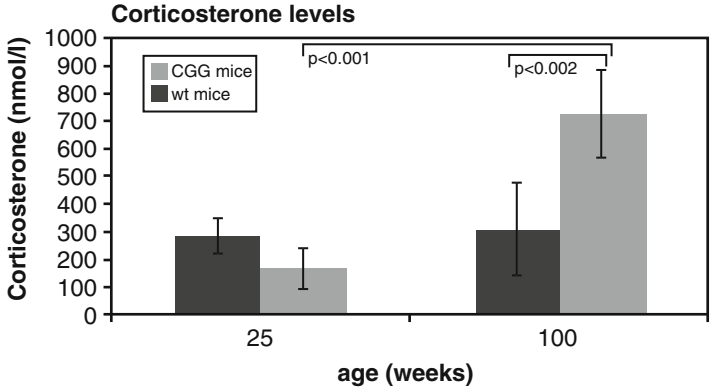

Fig. 8.3 Mean corticosterone levels in serum of exCGG and WT mice at 25 and 100 weeks of age. At 100 weeks significantly elevated levels of corticosterone are found in exCGG mice compared to WT mice

In addition, high percentages of intranuclear ubiquitin-positive inclusions could be observed throughout the amygdala. Thus the altered regulation of the HPA axis and the amygdala, along with increased stress hormone levels, may explain the increased psychopathology seen in male premutation carriers. Future clinical research to investigate the origin of the psychopathology should therefore be focused on the determination of salivary cortisol levels in premutation carriers.

Therapy and Mouse Models

No effective therapy is currently available for FXTAS as existing treatments are symptomatic. As a first step to develop molecular therapeutic interventions and to produce pharmacological agents to ameliorate or reverse neuropathology in patients with FXTAS, we need to better understand the disease pathogenesis, its underlying molecular mechanistic basis, and expectations for reversibility of disease. Recent advances have been made by the use of transgenic mouse models in which the expression of a $(CGG)_{90}$ repeat can be induced by doxycycline (dox) (Hukema et al. 2014). In this model, the $(CGG)_{90}$ repeat was taken out of the context of the *Fmr1* gene and placed under control of a tetracycline response element (TRE) and coupled to an eGFP reporter. Ubiquitous expression of the $(CGG)_{90}$ repeat under control of an hnRRNP-rtTA driver resulted in the early death of these mice within 5 days of dox induction. The livers of these mice showed mitochondrial dysfunction and increased apoptosis. In this study no inclusions could be observed, suggesting the free CGG RNA may exert a toxic effect. The early death of the mice did not allow for further studies in the brains of these mice, since expression was too brief to induce neuropathology. Subsequent studies were designed with a brain specific driver: PrP-rtTA (Hukema et al. 2015). In these mice, dox-induced expression of the $(CGG)_{90}$ repeat resulted in formation of intranuclear inclusions positive for both ubiquitin and FMRpolyG after 8 weeks of expression. Additionally, these inclusions increased in number and size over time. The main advantage of this inducible model system is that transgene expression can be turned off which may halt or reverse disease pathogenesis. In fact, stopping $(CGG)_{90}$ expression after 8 weeks (on dox) and followed by a 12-week wash-out period (off dox) without expression resulted in a significant decrease of the size and number of these inclusions. In addition, performance on visuomotor tests (i.e., optokinetic response and vestibulo-ocular response) that was impaired in dox-treated mice, improved after $(CGG)_{90}$ expression was stopped by removal of dox (Hukema et al. 2015). These results indicate for the first time that the disease process can be halted and may be reversible if expression of the expanded CGG repeat can be reduced.

These results also suggest that an early therapeutic intervention might be beneficial for FXTAS patients. This inducible mouse model will also allow to further study the role of different brain regions and/or cell types in FXTAS pathology when using different dox-inducible transgenic mouse lines.

Drosophila *Model of FXTAS*

Drosophila has provided a powerful genetic system to elucidate fundamental cellular pathways. In recent years *Drosophila* has been used to study molecular mechanisms of several human neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases (Bilen and Bonini 2005). Studies in *Drosophila* can be carried out to understand the normal function of proteins involved in disease, as well as to study the effects of targeted expression of human mutant genes in the fly. Studies utilizing *Drosophila* models of human disease have provided new insights into the normal functions of such disease proteins and allowed for the development and validation of genetic or pharmacological therapies to prevent or delay neurodegenerative disease. To determine and further study the molecular pathogenesis of FXTAS, a fly model of FXTAS has been developed.

Development of FXTAS Drosophila Model

In order to study the cellular mechanisms of FXTAS, transgenic flies were made to overexpress a human genomic *FMR1* DNA fragment isolated from a premutation carrier. The DNA fragment contained 90 CGG repeats with a downstream EGFP coding sequence used as a reporter system. In this model, different promoters were applied to drive the transcription of the transgene in different tissues (Jin et al. 2003). Overexpression of $(CGG)_n$-EGFP ($n = 60$ and 90) in the retina led to a progressive neurodegeneration of the eye from day 1 to day 30, including loss of pigmentation and dysmorphology of ommatidia. Targeted expression of $(CGG)_{90}$-EGFP in pan-neuronal cells was lethal. Ubiquitous expression of $(CGG)_{90}$-EGFP in embryos was also lethal at the late larval stage (Jin et al. 2003). In contrast, expression of the $(CGG)_{90}$-EGFP at the same level as in neuronal cells did not show apparent toxic phenotypes in epithelial cells, suggesting differential sensitivity to the $(CGG)_{90}$ RNA toxicity between neuronal cells and epithelial cells. The toxicity of the fragile X premutation rCGG is dosage- and repeat length-dependent. Higher levels of expression and longer repeats cause more severe toxicity to neuronal cells. Thus, the combination of CGG repeat length and abundance of the *Fmr1* messages may determine the development of neurodegeneration observed in the fly model of FXTAS. Development of the FXTAS fly model also enabled the demonstration that the fragile X premutation rCGG repeat itself was sufficient to cause neuronal cell death, strongly supporting an RNA-mediated neurodegeneration mechanism.

Molecular Findings

Overexpression of fragile X premutation rCGG repeats in fly eyes induced the formation of inclusions in both nuclei and cytoplasm (Fig. 8.4), which is similar to the findings in the FXTAS knock-in mouse models (Jin and Warren 2003; Willemsen

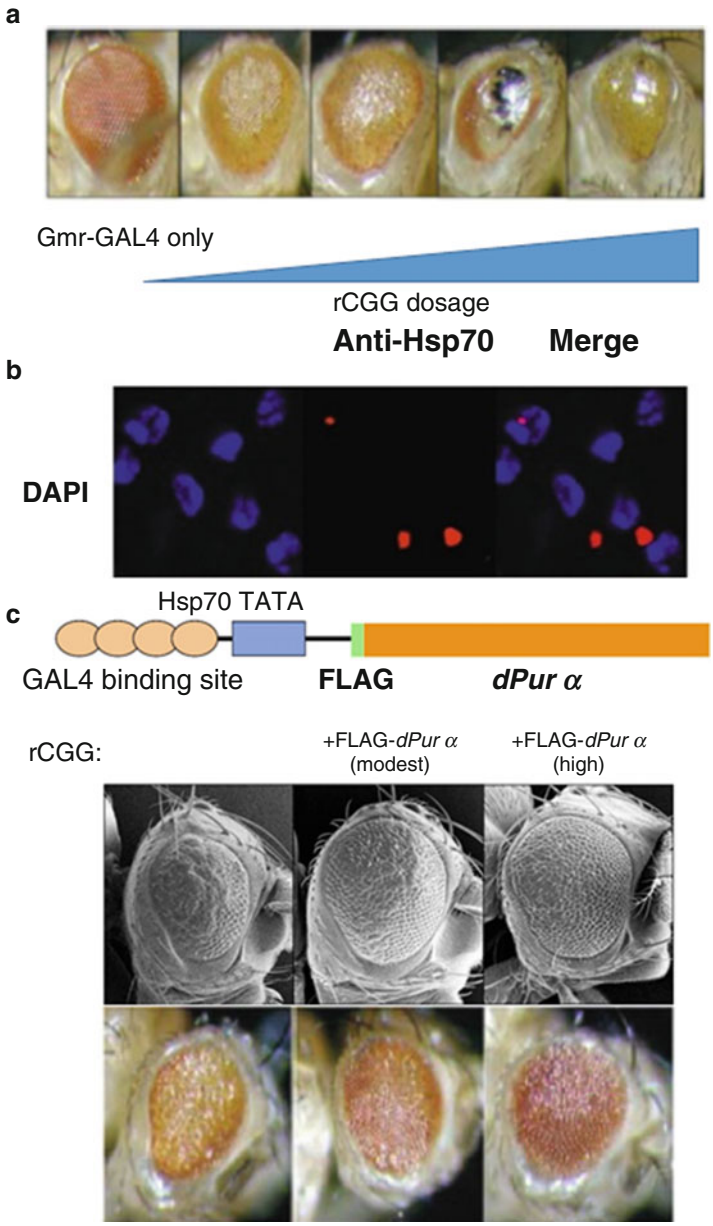

Fig. 8.4 (**a**) Fragile X premutation rCGG repeat results in induction of rCGG dosage-dependent neuronal cell death in fly eyes. (**b**) Fragile X premutation rCGG repeat results in induction of formation of Hsp70-positive inclusions in fly eyes. (**c**) Co-expression of rCGG-binding protein Pur α results in suppression of rCGG-mediated neurodegeneration

et al. 2003). The inclusions were positive for ubiquitin, Hsp70 chaperone, and proteasomal proteins. The presence of ubiquitin and proteasome complexes in the inclusions indicates a role for the protein degradation pathway in the pathogenesis of the FXTAS. In the fly, co-expression of both Hsp70 and Hsp40 chaperones suppressed the rCGG-induced neurodegeneration. In contrast, co-expression of the mutant Hsp70 with amino acid substitutions in the ATP-binding domain enhanced the neurodegeneration phenotypes. Hsp70 chaperone is believed to be a universal suppressor of multiple human neurodegenerative models caused by mutant proteins through the refolding of misfolded proteins (Chan et al. 2002; Cummings et al. 2001; Fernandez-Funez et al. 2000; Kazemi-Esfarjani and Benzer 2000; Warrick et al. 1998). It has also been suggested that Hsp70 may function as a critical modulator of the toxicity conferred by pathogenic RNAs such as rCGG-containing RNAs, in addition to its activity on misfolded proteins.

Given that the fragile X premutation rCGG repeat itself is sufficient to cause neurodegeneration in *Drosophila*, it has been hypothesized that specific rCGG binding may sequester rCGG repeat-binding proteins from their normal function (Jin and Warren 2003). Using biochemical and genetic approaches, three proteins, Pur α, hnRNP A2/B1 (two protein isoforms from one gene), and CUGBP1, were found to bind rCGG repeats either directly (Pur α and hnRNP A2/B1) or indirectly (CUGBP1, through the interaction with hnRNP A2/B1) (Jin et al. 2007; Sofola et al. 2007b). These proteins are RNA-binding proteins and have been shown to play a role in transcription, mRNA trafficking, splicing, and translation. Pur α and hnRNPA2/B1 have been shown to directly interact with rCGG. However, the interaction of CUGBP1 with rCGG specifically requires an association with hnRNP A2/B1. Furthermore, both Pur α and hnRNP were found in the inclusions of both human and fly tissues, suggesting that overexpression of the fragile X premutation rCGG repeats can sequester the rCGG-binding proteins from their normal cellular function(s) and cause neurodegeneration (Iwahashi et al. 2006; Sofola et al. 2007b). This idea is further supported by the fact that overexpression of either Pur α or hnRNP A2/B1 ameliorated neurodegeneration in the fly model of FXTAS. Interestingly, Pur α knockout mice appear normal at birth, but develop severe tremor and spontaneous seizures at 2 weeks of age, possibly due to substantially lower numbers of neurons in the hippocampus and cerebellum (Khalili et al. 2003). More recently, Tan et al. (2012) showed that a specific retrotransposon, *gypsy*, was activated in the rCGG fly model of FXTAS. This activation of *gypsy* may, in fact, be modulated by hnRNP A2/B1 through its interaction with heterochromatin protein 1 (HP1).

Several groups have been using *Drosophila* to identify novel therapeutic targets for FXTAS. Qurashi and colleagues (2012), using a chemical screen of the FXTAS *Drosophila* model, identified several phospholipase A_2 (PLA_2) inhibitors which may be associated with premutation rCGG-associated neurodegeneration. In subsequent testing, inhibition of PLA_2 activity was shown to attenuate or reverse rCGG-associated lethality and improve locomotor deficits in the FXTAS flies. Lin et al. (2013) showed that application of rapamycin could suppress neurodegeneration in flies expressing 90 CGG repeats, suggesting mTOR and its downstream molecules may also be potential therapeutic targets for FXTAS. Todd and colleagues (2010) found that overexpression

of histone deacetylases (HDAC) suppresses the neurodegeneration induced by CGG repeats in the FXTAS fly. Furthermore, treatment with histone acetyltransferase (HAT) inhibitors represses *FMR1* mRNA expression which was sufficient to control mRNA levels in premutation cell lines and extend the lifespan of the FXTAS flies. Similarly, overexpression of DCGR8 was found to rescue CGG repeat-induced neuronal death in the FXTAS fly, which was also confirmed in the exCGG mice (Todd et al. 2013). In another study, the ALS-associated RNA-binding protein TAR DNA-binding protein (TDP-43) was identified as a suppressor of CGG repeat-induced neurotoxicity in the FXTAS fly model (He et al. 2014). Reduced expression levels of TDP-43 have been reported in both fly and mouse models of FXTAS (Galloway et al. 2014). Furthermore, expression of wild-type TDP-43 in CGG90 *Drosophila* was sufficient to suppress neurodegeneration (Galloway et al. 2014). Taken together, the development and use of a fly model of FXTAS has facilitated the identification of several key rCGG-binding proteins thought to be involved in the molecular pathogenesis of FXTAS and may lead to the development of novel therapeutic targets.

RNAi Pathway and RAN Translation

It has been suggested that microRNA pathways might be involved in the processing of RNAs with lengthy triplet repeats into small RNAs (Handa et al. 2003; Krol et al. 2007; Ladd et al. 2007). Interestingly it has been reported that co-expression of both rCGG and rGCC in *Drosophila* could reverse their independent toxicities through reducing the levels of their transcripts (Sofola et al. 2007a). Although only a small portion of the transcripts (triplet repeats and flanking sequences ~300 bp) is complementary, the formed partial dsRNA could still stimulate RNAi pathways to cleave the dsRNAs. This is supported by the fact that the silencing of the triplet repeat RNAs occurs in an Argonaut 2 (AGO2)-dependent manner (Sofola et al. 2007a). This suggests a potential therapeutic strategy using complementary RNAs to target mutant mRNAs that carry lengthy triplet repeats. In addition, all triplet repeats, such as CAG, CUG, CGG, and GCC, may form internal hairpin loops, structures similar to the double-stranded RNA hairpins. These hairpin structures have been shown to be cleaved into shorter repeats by Dicer, a member of the ribonuclease III family that is a core component of the RNA interference machinery, by a mechanism similar to the processing of microRNA precursors (Handa et al. 2003; Krol et al. 2007; Sofola et al. 2007a). These shorter repeats, at least for CAG and CUG repeats generated from the long repeat hairpins, have been shown to trigger downstream transcriptional silencing effects via the RNAi pathway (Krol et al. 2007). Furthermore, it has been shown that the mutant transcripts containing lengthy triplet repeats can be highly and specifically silenced by synthetic oligonucleotides composed of complementary repeats (Krol et al. 2007). Sequestration of specific rCGG repeat-binding proteins could also lead to aberrant expression of selective miRNAs, which may modulate the pathogenesis of FXTAS by posttranscriptionally regulating the expression of specific mRNAs involved in FXTAS (Tan et al. 2012).

Increasing efforts are being made to apply RNAi technology to the development of new therapies for many diseases, including diseases of the central nervous system (for review De Fougerolles et al. 2007). Thus, the use of small interfering RNAs to silence mutant transcripts containing expanded trinucleotide repeats could represent a new approach to development of rational and effective treatments for FXTAS.

Additionally, the CGG repeats can trigger repeat-associated non-AUG initiated (RAN) translation of a cryptic polyglycine-containing protein, FMRpolyG. This polyglycine-containing protein was found in a Drosophila model of FXTAS and in the inclusions in postmortem brains of FXTAS patients and in the brains of exCGG mice. In *Drosophila*, this CGG repeat toxicity can be suppressed by eliminating RAN translation and enhanced by increased polyglycine protein production, suggesting a role for FMRpolyG in neurodegeneration in FXTAS (Todd et al. 2013).

Summary

The development of mouse and *Drosophila* models of FXTAS has facilitated cellular studies on the underlying molecular basis of this neurodegenerative disease. Such studies have provided critical information about the molecular events that occur with the onset and progression of the disorder, including new insights into the role of RNA toxicity in the pathophysiology of FXTAS. These are important contributions because of the difficulties, both logistical and ethical, in carrying out the necessary research in humans. For example, the development of brain pathology associated with FXTAS can be studied over the relatively brief lifetime of mice and flies, whereas similar studies in humans can only be carried out in extremely limited *postmortem* brains that only allow for end-stage studies of disease pathology. In addition, the existing models offer new opportunities to explore the relationship between the number of repeats and disease progression and in understanding RNA gain-of-function effects in the pathogenesis of FXTAS. Recent work using Drosophila models has also aided in the identification and testing of potential therapeutic targets for FXTAS. While neither the mouse nor *Drosophila* are perfect models, they do allow for the careful study of most of the key features of FXTAS. Continued research using these models should provide the critical information that will be needed for the development and evaluation of effective therapeutics for FXTAS.

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Chapter 9
Treatment and Management of FXTAS

Deborah A. Hall, Maureen A. Leehey, Elizabeth Berry-Kravis, and Randi J. Hagerman

Abstract Fragile X-associated tremor/ataxia syndrome (FXTAS) affects older adult carriers of the *FMR1* premutation and can be associated with a broad array of clinical symptoms and presentations including tremor, ataxia, parkinsonism, executive function disturbance and dementia, psychiatric symptoms of anxiety, depression and disinhibition, peripheral neuropathy, autonomic dysfunction, hormonal dysfunction, and pain syndromes. Although controlled trials have not demonstrated efficacy for FXTAS symptoms, there is information available regarding symptomatic treatments. Treatment is generally supportive, directed at component symptoms that are most problematic, and makes use of evidence regarding effectiveness of medications and other interventions for treatment of disorders that have phenotypic overlap with FXTAS. This chapter summarizes available treatments and supports that can be helpful for persons with FXTAS.

Keywords Treatment • Tremor • Ataxia • Parkinsonism • Memantine • Therapy • Deep brain stimulation

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General Approaches to Management

FXTAS is an inherited degenerative disorder that affects aging persons and is associated with an array of neurological symptoms and medical conditions. The complexity of the disorder requires the treating clinician to be cognizant of the broad and variable nature of FXTAS. For a detailed description of clinical manifestations, see Chaps. 1 and 3. Management of this multifaceted disorder requires a comprehensive approach, as outlined in Table 9.1.

Unfortunately, there is no specific treatment for FXTAS that is targeted to the underlying pathogenic mechanism of excess *FMR1* mRNA or other proposed mechanisms of disease. Furthermore, due to the recent recognition of the disorder and relatively few identified cases, as yet there have been few therapeutic clinical trials conducted in FXTAS. In a retrospective study of treatment in FXTAS, 56 patients with FXTAS completed a questionnaire to determine if any medications had been effective for neurological symptoms (Hall et al. 2006). This was followed by a review of medical records regarding treatment of their neurological symptoms. The study reported that 60 % were not on medications for their neurological symptoms (Hall et al. 2006). Those individuals with definite or probable FXTAS were more likely to be on medications than those with possible FXTAS, and 30 % of patients with possible or probable FXTAS were taking medications for motor signs (tremor, ataxia, or parkinsonism). The questionnaire study design may have underestimated reported effectiveness due to small sample sizes, cognitive impairment, and lack of insight into some of the symptoms of the disease.

Effective management of FXTAS requires the clinician to be knowledgeable about the whole phenotype and to consider the many symptoms that may be responsive to treatment with medication and other therapies and strategies. While there is no effective treatment that halts or slows the disorder, there has been some success in reducing a number of its disabling and distressing symptoms. Table 9.2 summarizes the categories of disease and symptoms that may be affecting each individual with FXTAS and types of therapy that may be beneficial. The therapeutic strategies we present are based on the questionnaire (Hall et al. 2006) and current experience from our centers and others evaluating and treating FXTAS.

Table 9.1 Management of FXTAS

Treat specific symptoms, e.g., neurological, psychiatric
Refer to appropriate specialists: neurology (e.g., movement disorders), psychiatry, gerontology, urology; physical, occupational, and speech therapy
Monitor for and treat comorbidities
Genetic counseling for the patient and family
Avoid medications and conditions known that may worsen symptoms if possible

Table 9.2 Summary of the symptomatic treatment of FXTAS

Action tremor
Beta-blockers, primidone, topiramate, benzodiazepines, other medications, occupational therapy, thalamic deep brain stimulation
Cerebellar ataxia
Amantadine, riluzole, buspirone, other medications, physical therapy
Parkinsonism
Dopaminergic medications, anticholinergics, beta-blockers for tremor, physical therapy
Painful neuropathy
Gabapentin, pregabalin, duloxetine, lidocaine patches or cream
Autonomic dysfunction
Urinary urgency and frequency[a]
Tricyclic antidepressants, antimuscarinics, botulinum toxin injection
Constipation
↑ fluids and fiber, stool softeners, other standard treatment
Impotence
Testosterone replacement if indicated, urology referral
Orthostatic hypotension
↑ fluids and salt, elevate head of bed, constriction stockings, small frequent meals, fludrocortisone, midodrine, pyridostigmine
Cognitive impairment
Anticholinesterases ± memantine, problem-solving therapy for executive dysfunction
Anxiety, agitation, depression
Selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors (especially venlafaxine)
Dysphagia
Swallowing evaluation and therapy
Fibromyalgia, chronic fatigue, irritable bowel syndrome
Exercise, patient education, cognitive-behavioral therapy, antidepressants, integrative medicine, and other referrals
Thyroid disease
Evaluation and treat as indicated

[a]Urological referral is indicated before initiating therapy since patients with FXTAS may have urinary retention

Treatment for the Movement Disorders in FXTAS

Clinical Trial on Memantine in FXTAS

To date, there has been only one clinical trial conducted in FXTAS. A randomized, double-blind, placebo-controlled, 1-year trial of memantine was conducted in men and women with FXTAS (Seritan et al. 2014). Anecdotal reports of memantine, an NMDA receptor antagonist, suggested that the medication might improve neurological and cognitive symptoms of FXTAS. Primary outcome measures in the study

were the Behavioral Dyscontrol Scale (BDS) and intention tremor severity on the CATSYS. The CATSYS is a set of computer-assisted diagnostic instruments that measure intention tremor, postural tremor, postural sway, manual coordination, and reaction time (Despres et al. 2000). Ninety four patients were randomized and 43 and 45 patients, respectively, received memantine or placebo. Intention-to-treat analysis of the 88 subjects in the study showed no improvement of BDS score (16.1 ± 5.4 vs. 15.7 ± 4, $p = 0.727$) nor intention tremor severity (1 ± 0.7 vs. 1.9 ± 2.2, $p = 0.047$) (Seritan et al. 2014). The authors of the trial report that limitations of the study included a moderate sample size, fewer subjects with late FXTAS, using only the CATSYS to record tremor, and psychotropic medication changes during the 12 months of the study. A subgroup of the FXTAS patients ($n = 41$) in the memantine controlled trial were studied with event related potential (ERP) studies at baseline and after 1 year of treatment (Yang et al. 2014). Memantine treatment improved both cued recall memory and the N400 repetition effect amplitude in the ERP studies compared to placebo. Future studies that combine memantine with additional agents may be warranted based on these results (Yang et al. 2014).

Treatment of Tremor

There are few studies published describing medications that are effective tremor in FXTAS. In a case report of single FXTAS patients with kinetic tremor, levetiracetam at 1000 mg/day was effective to reduce the intention tremor and improve handwriting (Saponara et al. 2009). Of the subjects with FXTAS receiving therapy for kinetic tremor in the retrospective questionnaire study, three out of six reported mild to moderate improvement on primidone, three out of eight had moderate improvement in tremor on beta-blockers, and two out of eight had moderate improvement on benzodiazepines (Hall et al. 2006). This suggests that medicines helpful for essential tremor may also be effective in some patients with FXTAS. One subject had improved tremor on memantine, which was prescribed for cognitive decline. There was no improvement in tremor for two subjects on gabapentin.

In the initial case studies utilizing thalamic deep brain stimulation (DBS) for management of tremor in individuals with FXTAS (Leehey et al. 2003; Peters et al. 2006), results were mixed. Of three patients with DBS placed, two patients had substantial improvement in tremor, but speech and ataxia worsened in one and remained stable in the other. Neither of these individuals showed worsening of executive or cognitive function. A subsequent case report of a FXTAS man with nucleus ventralis intermedius (VIM) of the thalamus DBS placement described immediate progression of ataxia, cognitive decline, dysarthria, and apraxia (Mehanna and Itin 2014). More recent studies suggest that some FXTAS patients can have sustained improvement with DBS into the VIM. Three FXTAS men showed improvement for up to 2 years after surgery in both tremor and gait (Weiss et al. 2015). DBS with stimulator placement into the ventro-oralis posterior thalamic nucleus and zona incerta in a FXTAS man with postural and action tremor resulted in significant and stable control of tremor for 20 months (dos Santos Ghilardi et al. 2015). In addition, his ataxia

improved by 50 %. The authors postulated that avoidance of stimulation of the VIM and restricted electrical fields of bipolar stimulation could be responsible for the long-term improvement. Persons with preexisting cognitive dysfunction tend to worsen with DBS (Aybek and Vingerhoets 2007), and the worsening of ataxia and cognition noted in some FXTAS patients is also a significant concern (Hagerman et al. 2012). Thus, careful evaluation is needed before considering DBS, and candidates for this procedure would be limited to individuals with FXTAS and disabling medically resistant tremor and minimal cognitive dysfunction.

Beta-blockers and primidone are commonly used to treat essential tremor (ET) and would be the likely candidates for therapy for tremor in FXTAS. Information on the effectiveness of treatment may be borrowed from the ET treatment literature, albeit recognizing that medication efficacy in FXTAS may not be the same. Propranolol, a β-adrenergic blocker, is an effective medication for the treatment of ET (Caccia et al. 1989; Calzetti et al. 1990). However, these medications are contraindicated in patients with asthma, second-degree AV block, congestive heart failure, and insulin-dependent diabetes, with fatigability, impotence, lightheadedness, sedation, and even depressive symptoms as common side effects. Sotalol and atenolol (other beta-blockers that may have less side effects than propranolol) may also be effective (Zesiewicz et al. 2011). Primidone has also been shown to be effective in placebo-controlled studies in ET (Koller and Royse 1986), with its anti-tremor effect attributed to its metabolite phenobarbital (Sasso et al. 1991). It is started at low doses to minimize side effects, such as nausea, vomiting, sedation, and confusion. Topiramate is a possible third-line choice for ET (Connor 2002), although this medication may be less ideal given the cognitive side effects in FXTAS patients. Gabapentin has been shown to be effective in small studies in both improving activities of daily living and tremor rating scale scores (Ondo et al. 2000). Benzodiazepines such as alprazolam or clonazepam may also be effective in some patients (Zesiewicz et al. 2011; Gunal et al. 2000). Because tremor is aggravated by anxiety or stress, which are common in *FMR1* premutation carriers, benzodiazepines may help to decrease anxiety and thus reduce tremor secondarily.

Treatment of Ataxia

Most of the gait ataxia in FXTAS is due to cerebellar dysfunction. Gait ataxia improved in one patient who was taking amantadine in the questionnaire study (Hall et al. 2006) and in two additional patients reported by Jacquemont et al. (2004). Amantadine, an NMDA receptor antagonist, has been shown to help ataxia in patients with olivopontocerebellar atrophy (which is similar in phenotype to FXTAS) in a small clinical trial (Botez et al. 1996). Ataxia improved in a 65-year-old man with FXTAS who was treated with varenicline as part of a smoking cessation program (Zesiewicz et al. 2009). He improved from requiring a walker to ambulate to not needing a walking aid, worsened when the varenicline was discontinued, and improved when it was restarted. A randomized, double-blind placebo-controlled trial showed improvement in cerebellar ataxia after treatment with riluzole relative

to placebo (Ristori et al. 2010). One subject in the trial had FXTAS, was in the riluzole group, and showed improvement in ataxia. Gait difficulties may also be caused by parkinsonism, extensive cerebral hemispheric white matter lesions, and peripheral neuropathy. Dopaminergic medications were felt to be beneficial in some parkinsonian persons with gait ataxia (Hall et al. 2006). Buspirone was tested in patients with cerebellar ataxia and found to have improved ataxic kinetic score (Trouillas et al. 1997). Gabapentin has been reported in an open trial to improve cerebellar signs in patients with cerebellar cortical atrophy (Gazulla et al. 2003). Gabapentin may be an especially good option for FXTAS patients with neuropathy and nerve pain. A recent consensus paper on the management of cerebellar ataxia suggests that acetyl-DL-leucine may be safe and improve gait ataxia and dysmetria in patients with cerebellar ataxia of various etiologies (Ilg et al. 2014). However, this compound is not routinely used in the clinic and additional research specifically in FXTAS may be needed.

Treatment of Parkinsonism

In the retrospective questionnaire study, rest tremor was not evaluated exclusively, but parkinsonism (rest tremor, slowness, or stiffness) in patients with FXTAS improved on carbidopa/levodopa in four of ten cases, pramipexole in three of six cases, and eldepryl in one patient (Hall et al. 2006). In FXTAS patients with parkinsonism and gait abnormalities, subjective improvement in gait was seen on carbidopa/levodopa, dopamine agonists, and eldepryl (Hall et al. 2006). Although parkinsonism is considered a minor criteria for FXTAS, some patients are dopamine responsive, similar to patients with primary Parkinson disease. Because motor fluctuations and dyskinesia have not been reported in patients with FXTAS, dopaminergic therapy should be considered if parkinsonism is present and problematic in a patient with FXTAS. Introduction of carbidopa/levodopa must be done with care, however, as worsening of motor control and autonomic and/or cognitive symptoms can occur and hallucinations and significant lethargy can be seen in some individuals with FXTAS. In FXTAS, the rest tremor is not usually disabling and thus by itself may not require treatment. Anticholinergics, such as trihexyphenidyl and benztropine, may be useful in reducing PD rest tremor, but are often not well tolerated in the elderly due to adverse effects, including short-term memory dysfunction, urinary retention, and constipation.

Rehabilitative Therapies for Motor Dysfunction in FXTAS

Physical therapy can be helpful for improving strength and gait in treatment of persons with FXTAS (Hall and O'Keefe 2012). Quantitative gait analysis and even a routine gait evaluation through physical therapy can help the patient understand

what activities they can be independent of and when they need support in the form of a cane or walker and thus prevent falls. Significant improvements in balance, gait ataxia, and activities of daily living have been shown after 4 weeks of intensive physical therapy in patients with degenerative cerebellar ataxia of various causes (Ilg et al. 2014). These improvements persist for a year when the participants maintained a daily home exercise program. Body weight supported treadmill training for gait deficits and computerized dynamic posturography with biofeedback retraining devices are relatively newer options that could be considered for FXTAS patients (Hall and O'Keefe 2012). Occupational therapy may be beneficial in improving performance of functional tasks. OT has been shown to improve disability scores and quality of life in after 15 sessions in patients with spinocerebellar ataxia type 3 (Silva et al. 2010). Assistive technology to aid feeding and computer use in patients with intention tremor may be useful (Feys et al. 2001; Surdilovic and Zhang 2006). Increasing the inertia or viscoelastic resistance of a limb may dampen kinetic tremor. Interventions might include trunk or limb loading with weights or use of trunk vests and trunk stability (Morrice et al. 1990; Gibson-Horn 2008; Marsden and Harris 2011). *FMR1* premutation carriers may be at higher risk of hearing loss and referral to audiology and provision of hearing devices should be considered if warranted.

Treatment for Neuropathy and Pain in FXTAS

Pain is a common problem in patients with FXTAS. Neuropathic pain, particularly in the lower extremities, is seen in both men and women with FXTAS. Neuropathic pain can be difficult to treat and does not often respond well to nonsteroidal anti-inflammatory agents. Many patients, however, can derive meaningful symptomatic relief from other available pharmacologic treatments, including anticonvulsants, antidepressants, and topical analgesics (Gilron et al. 2006). Specific medications that have been shown to be beneficial in other causes of painful neuropathy include gabapentin, pregabalin, or duloxetine. These latter medications thus would be expected to be beneficial in FXTAS. Some women with FXTAS have been diagnosed with concurrent fibromyalgia, which is associated with pain (Coffey et al. 2008; Leehey et al. 2011). Initial treatment for these symptoms should include non-pharmacological approaches such as exercise, patient education, and cognitive-behavioral therapy. The most effective pharmocotherapies include antidepressants, anticonvulsants, or muscle relaxants (Goldenberg et al. 2004). If these treatments are not successful for management of pain, patients should be referred to specialty clinics, such as rheumatology, for further management and if needed can be referred to a pain rehabilitation program. Massage therapy can be helpful at reducing chronic stress, and acupressure/pressure point massage has been shown to be efficacious for pain reduction in fibromyalgia (Tsao 2007). For the elderly, massage therapies can represent an alternative to treatment with pharmacological agents, especially when side effects of medication are a concern or when there are drug interactions, or massage therapy can be complementary or additive to pharmacotherapy.

Treatment for Autonomic Symptoms in FXTAS

Autonomic dysfunction in FXTAS may include impotence, orthostatic hypotension, urinary frequency or incontinence, and bowel incontinence (in the later stages) (Jacquemont et al. 2003). Most men with average fluid intake urinate about every 3 h, but this may become as frequent as every 20 min in severely affected individuals with FXTAS. Micturition can be associated with difficulty starting the stream, emptying the bladder, and dribbling. Although detailed urological studies have not been conducted in FXTAS, hyperactive detrusor activity is possible since some individuals with FXTAS respond well to small doses of tricyclic antidepressants or to muscarinic receptor antagonists. In patients refractory to this therapy, there may be poor bladder contractility or sphincter dyssynergia. In one patient with FXTAS, a more effective treatment was botulinum toxin injection into the submucosal lining of the bladder, carried out by cystoscopy with a small diameter cystoscope under local anesthesia (Hagerman et al. 2008). This procedure was well tolerated and mild hematuria resolved within 3–5 days. Antibiotics were used postinjection and infections are considered rare with this procedure. The effects of the botulinum can last from 3 to 4 months or longer. Rare patients with FXTAS will develop an inability to urinate, and self-catheterization will need to be taught.

Constipation is common in the elderly and can be a problem in FXTAS. Treatment of chronic constipation in these patients would begin with dietary changes, high fiber supplements, and over-the-counter medications such as stool softeners and milk of magnesia to encourage normal peristalsis. Laxatives can be used in more severe cases. Side effects, particularly diarrhea, may require modifying laxative regimens. The rationale behind a bowel regimen is to slowly train the bowel to constrict down to a more normal size. Further studies of this problem in FXTAS may help to clarify treatment protocols.

Orthostatic hypotension is seen in a subset of patients with FXTAS and can be treated nonpharmacologically with increased fluid and salt intake, elevating the head of the bed at night, use of Jobst stockings, and eating frequent small meals. Medications effective for orthostatic hypotension include mineralocorticoids such as fludrocortisone to expand blood volume and improve perfusion and orthostatic symptoms. Midodrine, an alpha-1 adrenergic agonist which increases blood pressure, may also be used. This medication may cause vasoconstriction, pupil dilation, a “hair standing on end” sensation, and itching or paresthesia of the scalp. Patients need to be warned not to lie flat for 4 h after a dose, as dangerously high blood pressure can occur. Doses can be increased quickly until a response occurs or a dose of 30 mg/day is attained (Wright et al. 1998). Midodrine has the advantage that it does not cross the blood–brain barrier and therefore does not have CNS side effects. Midodrine has been shown to be helpful in controlled trials (Wright et al. 1998); however, some patients require a combination of both midodrine and fludrocortisone. Orthostasis can also be treated with pyridostigmine (Gales and Gales 2008), a reversible acetylcholinesterase inhibitor that enhances sympathetic cholinergic signal transduction and thus increases peripheral vascular resistance and baroreceptor

sensitivity. This mechanism allows for improved orthostatic symptoms without exacerbation of supine hypertension. Droxidopa, recently approved, is an option in refractory cases (Kaufmann et al. 2014).

Treatment for Swallowing Difficulties

Swallowing difficulties are a common, typically late symptom in FXTAS. In patients with dysphagia, routine monitoring and therapy by a speech therapist may be useful to prevent aspiration and maintain communication with others (Revuelta and Wilmot 2010). Depending on the nature of the dysphagia, the patient can be educated regarding techniques to enhance proper delivery of the food to the stomach, including positional changes when they are swallowing. For patients with more severe problems, a modified diet may be necessary to avoid aspiration pneumonia. One individual with FXTAS and swallowing difficulties had an excellent response to pyridostigmine (Hagerman et al. 2008). Adverse effects of pyridostigmine treatment may occur due to stimulation of the parasympathetic nervous system via muscarinic cholinergic receptors and can include sweating, salivation, nausea, vomiting, diarrhea, and abdominal cramping.

Treatment for Dementia in FXTAS

Cognitive changes in patients with FXTAS include executive function deficits and memory problems (Grigsby et al. 2006), see Chap. 3. The cognitive changes progress at a variable rate and dementia develops in at least 50 % of men with FXTAS (Bourgeois et al. 2006). Although the profile of cognitive deficits in FXTAS appears to be different from that classically seen in AD, the use of medications that slow cognitive decline in other dementias may be warranted. Clinical intervention with psychopharmacological agents, as is typically done for other subtypes of dementia, appears advisable in FXTAS dementia and may serve to stabilize cognitive function, at least in the short term.

Donepezil and other cholinesterase inhibitors can be considered in patients with FXTAS and memory impairment. In the early months of treatment, memory function may be improved resulting in enhanced quality of life, even if the eventual trajectory of memory decline is not substantially changed. In the questionnaire study, slowing of cognitive decline was reported by family members in three out of nine patients with FXTAS treated with acetylcholinesterase inhibitors (Hall et al. 2006). Combination therapy of donepezil and venlafaxine over a 2-year duration has been reported to improve and stabilize the dementia and mood alterations (depression, anxiety, agitation, hostility, and irritability) in a case report of a FXTAS patient who had multiple neurological symptoms (Bourgeois et al. 2006). Other acetylcholinesterase inhibitors could be considered depending on the side effect profile and preferences of the patient

(for example, use of a patch rather than pills). The use of memantine is summarized earlier in this chapter.

Evaluation for other causes of dementia, particularly reversible contributing causes, such as hypothyroidism, B_{12} deficiency, B_6 deficiency, and folate deficiency, is essential, with vitamin supplementation in cases in which levels are low. Exercise has been found to be helpful for executive cognitive and depressive symptoms in Alzheimer's disease, and an exercise program that includes walking, strength, balance, and flexibility training may be therapeutic in FXTAS. This should be guided by the physical therapist for individuals with substantial motor dysfunction. Some patients with FXTAS have significant brain atrophy with dilation of the ventricles that is mistaken for normal pressure hydrocephalus (NPH) (Hall et al. 2005). However, surgery for presumed NPH has resulted in deterioration when performed in patients with FXTAS (Jacquemont et al. 2004). Support for family caretakers during the dementia process is crucial, and the decision to place a patient with FXTAS in a specialized center for the care of individuals with dementia may be necessary. Supportive help in the household is frequently needed prior to placement of the patient in a chronic care facility. Involvement of social workers with expertise in aged populations can greatly facilitate transitions to other living environments or finding resources for respite for caregivers.

Some patients with neurological disease, including FXTAS, do not tolerate major surgery with general anesthesia well and further deterioration in both motor and cognitive abilities may occur. It is unclear if there is a specific relationship to FXTAS, anesthesia, or the surgery itself. Elective surgery, therefore, should be considered cautiously in any patient with significant neurological disability, including patients with FXTAS.

Treatment for Psychiatric Symptoms in FXTAS

Psychiatric problems commonly seen in patients with FXTAS include anxiety, agitation, apathy, and depression (Bacalman et al. 2006). Both the anxiety and the depressive disorders associated with FXTAS may benefit from management with antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs) or serotonin–norepinephrine reuptake inhibitors (SNRIs) (Hall et al. 2006; Jacquemont et al. 2004). The selective serotonin–norepinephrine reuptake inhibitors (SNRIs; venlafaxine and duloxetine) may be effective for both depressive and anxiety symptoms and should be considered as their noradrenergic activity may be desirable. Both SNRIs need to be used with caution in renal failure. In the questionnaire study, anxiety improved in two out of six patients with FXTAS on venlafaxine and five out of eight patients on benzodiazepines (Hall et al. 2006). Due to the progressive nature of FXTAS and presence of dementia in some affected individuals, benzodiazepines (which may impair cognitive function) and tricyclic antidepressants (which are anticholinergic and thus can theoretically be problematic in patients with cognitive impairment) should be used cautiously (Gray et al. 1999; Oxman 1996). Intervention

with antidepressants for coexisting mood and anxiety disorders may, however, improve cognitive function due to improvement in the emotional state (Bourgeois et al. 2006) and two of six patients treated with venlafaxine reported slowing of cognitive decline (Hall et al. 2006). Individuals with dementia and secondary psychotic symptoms (e.g., delusions and hallucinations) may benefit from cautious use of atypical antipsychotic agents (Bourgeois et al. 2006).

Combined psychopharmacological approaches with antidepressants and antipsychotics along with medications to slow cognitive decline may be indicated. SSRIs with minimal drug–drug interaction profiles (e.g., sertraline, citalopram, escitalopram) are preferred for use in the elderly, but all medications in this class may be effective. Mirtazapine can be helpful for sedation and appetite stimulation, but it requires a reduced dose in renal failure. For psychotic symptoms, cautious doses of atypical antipsychotics are recommended with close follow-up. Quetiapine and clozapine are the best choices as they are unlikely to exacerbate underlying parkinsonism. Treatment with clozapine requires frequent blood draws to monitor for pancytopenia and has the potential to aggravate orthostatic hypotension. Thus, while it can be quite beneficial for psychosis and parkinsonism, quetiapine may be a better first choice.

Psychosocial Approaches to Cognitive and Psychiatric Problems in FXTAS

It is important to provide the patient and family with education and supportive intervention and it is helpful to deliver psychiatric care in the context of a multidisciplinary team. Since several members in the same family are often affected, intervention plans need to target the patient and the family, and consistent longitudinal follow-up is crucial.

Problem-solving therapy (PST) has been shown to improve depressive symptoms and functioning in elderly patients with executive dysfunction and depression, cognitively unimpaired depressed elderly, and younger adults with minor and major depression (Arean et al. 1993). Active intervention with a depression "care manager" may also improve outcomes, even in individuals with cognitive dysfunction (Steffens et al. 2006). By extension, caregiver well-being may also improve as symptoms of depression and anxiety in the patient become less problematic (Hagerman et al. 2008).

Psychological support for caregivers is an important issue to be addressed in the management of FXTAS. Women constitute the majority of caregivers for patients with FXTAS and 70 % of the caregiving population (Hagerman et al. 2008). Women were found to have significantly higher caregiver burden than male caregivers. Poor perceived physical health and more behavior disturbance in the patient were associated with higher levels of caregiver burden and depression (Gallicchio et al. 2002). Based on clinical experience depression seems to be very common in the wives of men with FXTAS and they may do well with supportive counseling and referral for treatment of

their depression. In a study of dementia caregivers' reaction to the death of their care recipient relative, 20% of caregivers experienced complicated grief along with high levels of depressive symptomatology (Schulz et al. 2006). Caregivers with high levels of pre-loss depressive symptoms and burden, who reported positive features of the caregiving experience, and cared for a more cognitively impaired patient were more likely to report complicated bereavement post-loss. The importance of caregiver support, however, was illustrated by the finding that caregivers who were enrolled in a psychosocial caregiver intervention designed to reduce depression and burden reported lower levels of complicated grief (Schulz et al. 2006).

Treatment of Hormonal Dysfunction

Recent studies have broadened our concept of FXTAS to include hormonal dysfunction. Inclusions have been documented in the anterior and posterior pituitary (Louis et al. 2006; Greco et al. 2007) and in the Leydig cells in the testicles that produce testosterone. Testosterone deficiency has been reported in five of eight premutation carriers that have been tested and may be related to the pituitary and Leydig cell involvement (Greco et al. 2007). Affected individuals may become aware of erectile dysfunction before the onset of other neurological signs. Because low testosterone can cause impotence, testosterone replacement may improve libido and sexual function in patients with FXTAS. Other problems associated with FXTAS which might improve from testosterone replacement include cognition, memory, energy level, and mood (Cherrier et al. 2003). Hormone replacement in patients with FXTAS would follow the same paradigm as with other hypogonadal states. Preferred regimens, used in other diseases, would include transcutaneous testosterone administration or intramuscular injection. Further studies of testosterone deficiency in FXTAS are warranted and the benefits and risks of testosterone replacement require study. Treatment with testosterone may increase the risk of prostate cancer, and careful follow-up with PSA levels and frequent examination are recommended if testosterone treatment is utilized. In addition to hormonal dysfunction and primary ovarian insufficiency seen in female carriers, hypothyroidism appears to be a frequent problem in females with the premutation. Thyroid dysfunction occurred in 50% of women with FXTAS in one study suggesting that routine thyroid function testing is indicated yearly for women with FXTAS (Coffey et al. 2008). Replacement is indicated for hypothyroidism, which can aggravate baseline emotional and psychiatric problems observed in premutation carriers (Devdhar et al. 2007).

Treatment of Other Comorbidities

Hypertension (HTN) was more common in men with FXTAS in a study that evaluated two cohorts of FXTAS men and controls (Hamlin et al. 2012). Both chart review and blood pressure measurements (HTN defined >140/90) in clinic were

utilized to define subjects with HTN. FXTAS men had an odds ratio of 3.22 (CI: 1.72–6.04) compared to controls. Because hypertension can lead to small vessel ischemia, exacerbating existing white matter disease from FXTAS itself, it is important to treat it early and aggressively.

Migraine headaches have been reported to be increased in premutation carriers compared to controls (Au et al. 2013). Migraines in premutation carriers often begin in the third or fourth decade and can persist and even worsen in individuals with FXTAS. Persistent migraines can also be associated with white matter disease and it is possible that persistent migraine may exacerbate white matter disease in FXTAS.

A careful history may reveal other comorbidities that may exacerbate FXTAS including substance abuse particularly alcohol or opioids (Muzar et al. 2014). In addition, sleep apnea is common in FXTAS and the hypoxia associated with sleep apnea can be treated with CPAP and oxygen (Hamlin et al. 2011). A sleep apnea study should be ordered if the history is suggestive of this problem.

Genetic Counseling

FXTAS is an inherited condition and because diagnosis of FXTAS nearly always implies that other individuals in the family will be at risk for *FMR1*-related disorders, genetic counseling is an important part of the treatment plan once a diagnosis is made. Many individuals are diagnosed with FXTAS as a result of diagnosis of fragile X syndrome in another family member, such as a child or a grandchild, and a genetic counselor may already be working with the family. Particularly if FXTAS is diagnosed without prior knowledge of *FMR1* mutations in the family, it is crucial that the male patient understand that all of his daughters will be *FMR1* carriers and are at risk for children with fragile X syndrome and themselves at risk for primary ovarian insufficiency. Women diagnosed with FXTAS must understand that all their children have a 50 % risk of being *FMR1* carriers or being affected with fragile X syndrome. There will also likely be numerous brothers, sisters, nephews, nieces, cousins, and other relatives who will be at risk for an *FMR1*-related disorder, making genetic counseling imperative so that the patient is provided with knowledge about this situation and recommendations on how to approach relatives with this information. The reader is referred to Chap. 10 for a thorough discussion of approaches to genetic counseling.

Future Treatments to Target the Underlying Mechanism of Disease in FXTAS

Development of therapeutic interventions that target the core molecular processes that result in FXTAS is essential. Such therapeutics would target the pathogenic trigger, the toxic expanded CGG repeat *FMR1* mRNA itself, or downstream

pathways that are altered as a consequence of the toxic mRNA expression. A conceptually simple approach would be to use antisense or RNA interference agents to reduce the pathogenic RNA itself; however, a difficulty inherent in this approach would be the general inability of these agents to cross the blood–brain barrier. Increased knowledge about proteins involved in the toxic response to the repeat-containing RNA and downstream pathways is expected to provide additional targets for interruption of the underlying molecular process through which FXTAS occurs.

Summary

There is currently no cure that can reverse the pathophysiology of FXTAS. However, there are a number of symptomatic treatments that may improve the quality of life of these patients. Identification of the disorder may be the first hurdle for individuals with FXTAS and referral to appropriate specialists may be very helpful, depending on the most problematic symptoms. As the underlying etiology of FXTAS is better delineated, neuroprotective agents or agents targeted at the underlying toxic RNA mechanism may be added to symptomatic therapy to slow down or stall the disease progression.

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Chapter 10
Clinical Manifestation and Management of FXPOI

Stephanie L. Sherman, Emily G. Allen, Jessica B. Spencer, and Lawrence M. Nelson

Abstract Fragile X-associated primary ovarian insufficiency (FXPOI) is among a family of disorders caused by the expansion of a CGG repeat sequence located in the 5′ untranslated region of the X-linked gene *FMR1*. About 20 % of women who carry a premutation have cessation of menses for at least 1 year prior to age 40, a 20-fold increased risk compared with the general population. Further, the frequency of women with the premutation attending reproductive endocrinology clinics for infertility is significantly increased compared with the carrier frequency expected in the general population, ~3 % compared with 1/150–1/250. This makes FXPOI the leading known inherited cause of idiopathic POI. Cross-sectional studies clearly show the health burden related to the clinical outcomes associated with FXPOI. Despite the significant reduction in reproductive life span and increased frequency of estrogen deficiency-related medical conditions among women with the premutation who are diagnosed with FXPOI, little is known about the underlying molecular etiology. In this chapter, we focus on describing the clinical manifestation of FXPOI, the risk factors that potentially lead to earlier onset and severity, and the clinical management that can help ameliorate symptoms.

Keywords Menopause • Primary ovarian insufficiency • Estrogen deficiency • Infertility • Repeat expansion disorder

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Overview and Focus

Reproductive health is a strong predictor of overall health and well-being. One marker of reproductive health in women is the age at natural menopause. The median age of menopause is ~51 ± 1 years, with 1 % of women experiencing menopause prematurely (Palacios et al. 2010). In the late 1990s and early 2000s, the *FMR1* premutation was established as an important genetic cause of premature ovarian failure (POF) (reviewed in Sherman 2000). At that time, the term POF was defined as 12 months of secondary amenorrhea before age 40 and the majority of studies were based on this phenotype. The clinical definition and the term used for the observed ovarian dysfunction have changed. The term primary ovarian insufficiency (POI) is clinically defined as 4 months of oligo/amenorrhea before age 40 with two follicle stimulating hormone (FSH) levels in the menopausal range (as defined by the measuring laboratory) 1 month apart (Nelson 2009). In the current field of fragile X, FXPOI is used to encompass the spectrum of reproductive outcomes that includes not only cessation of regular menses before age 40, but also occult indicators of impaired ovarian function, which are manifest by a reduced ovarian response to stimulation, but no alteration in menstrual cyclicity.

FXPOI significantly impacts a woman's quality of life. The most immediate and significant consequence of diminished ovarian function is reduced fertility (Allen et al. 2007; Streuli et al. 2009; Wheeler et al. 2014). If we define POI here as cessation of menses for at least 1 year prior to age 40, POI occurs in ~20 % of women with the premutation, about ~20 times higher than the general population (for reviews, see De Caro et al. 2008; Sherman 2000; Sullivan et al. 2011). Taking all women who carry the premutation, they go through menopause on average about 5 years earlier than those without the premutation (Murray 2000; Seltzer et al. 2012; Sullivan et al. 2005). Since diminished ovarian reserve precedes POI by 10 or more years, impaired fertility may be seen in women with the premutation before the more typical decline in fertility observed after age 35.

The premutation occurs at a significantly higher frequency among women with ovarian dysfunction (e.g., Barasoain et al. 2013; Karimov et al. 2011; Mallolas et al. 2001; Murray et al. 2014; Pu et al. 2014; Streuli et al. 2009; Tosh et al. 2014; Ye et al. 2014). In a recent study of over 2000 women who experienced menopause before the age of 46 years, the prevalence of the premutation was 2.0 % among women with cessation of menses prior to age 40, 0.7 % in those with cessation between 40 and 45 years, and 0.4 % in controls. Combining women with cessation of menses prior to age 46, the odds ratio for carrying the premutation was 2.4 (95 % confidence interval = 1.02–5.8; $p = 0.04$). In studies that separated isolated and familial cases of POI, about 3.2 and 11 % of women carried the premutation (reviewed in Sherman et al. 2007).

Notably, the state of early estrogen deficiency observed among women with POI has significant clinical consequences, including an increased risk for low bone mineral density, earlier onset osteoporosis and bone fractures (Gallagher 2007), impaired endothelial function (Kalantaridou et al. 2004), earlier onset of coronary

heart disease (Atsma et al. 2006), and increased cardiovascular mortality and overall mortality (e.g., Jacobsen et al. 2003; Mondul et al. 2005). In addition, women with an earlier age at menopause are reported to have more anxiety, depression, somatization, sensitivity, hostility, and psychological distress than women with normal ovarian function (e.g., Van Der Stege et al. 2008). Hypoestrogenism has cognitive consequences as well. Estrogen is a neuroprotective agent that plays an important role in brain functioning, and changes in estrogen levels during aging are associated with reduced cognitive function and an increased risk of Alzheimer disease (Janicki and Schupf 2010).

Thus, women with FXPOI are at risk for these clinical disorders associated with prolonged estrogen deficiency. But it is important to emphasize that they are also at risk for other premutation-associated symptoms, most importantly neurodegenerative symptoms associated with fragile X-associated tremor/ataxia syndrome (FXTAS). As such, they may be more vulnerable to effects of early estrogen deficiency. The clinical manifestation of FXTAS in general is discussed in Chaps. 1–4 and FXTAS and other premutation-associated disorders specifically in women are reviewed in Chap. 12. At this point, a natural history study has not been conducted to determine whether women with FXPOI are at a higher risk for FXTAS than those without FXPOI.

Little is known about the mechanism that underlies FXPOI. Other repeat expansion disorders, including FXTAS, complemented by model systems of these disorders, have provided important insights into the possible etiology underlying FXPOI. These are described in detail in Chap. 11. As noted, not all women with the premutation experience POI—some have complete cessation of menses in their 20s while others go through menopause at the typical age of 51 (reviewed in De Caro et al. 2008). Why there is such extensive variation in the clinical outcomes of the *FMR1* premutation is unknown. In this chapter, we will focus on the clinical manifestation of FXPOI, the risk factors that potentially lead to earlier onset and severity, and the clinical management that can help ameliorate symptoms.

Primary Ovarian Insufficiency

A woman's reproductive life is based on the complex orchestration of the hypothalamic-pituitary-ovarian (HPO) axis. During early ovarian development, oocytes are organized into functional units called follicles. Each follicle consists of a single oocyte and associated granulosa cells. A woman's maximum endowment of oocytes (about six to seven million) occurs early in gestation, around 16–20 weeks (Baker 1963; Houmard et al. 2001). Through a natural process of follicular atresia, only about one to two million oocytes remain at birth and only 300,000–400,000 remain at menarche (Baker 1963; Block 1952). Thus, in the natural processes of ovarian development and age-related decline in ovarian function, a woman's pool of oocytes starts to decline well before she achieves reproductive maturity.

During a woman's reproductive years, primordial follicles are recruited and mature through a hormonally regulated process. The result of this process is a single oocyte selected for monthly ovulation. Due to the monthly ovulation and continued follicular atresia, the number of primordial follicles declines until about 1000 remain and the menopause ensues (Faddy 2000; Richardson et al. 1987). Age-related changes in both the quantity and quality of oocytes/follicles are responsible for the typical features of the age-related decline in ovarian function in women. These include characteristic hormonal fluctuations (elevated gonadotropins and decreased ovarian hormones), menstrual cycle changes (amenorrhea, oligomenorrhea, and dysfunctional uterine bleeding), diminished fertility and higher rates of twinning, aneuploidy and miscarriage.

The term "ovarian reserve" refers to a woman's remaining fertility potential attributable to her ovaries. However, this term is misleading and scientifically inaccurate because the biological measures to assess "ovarian reserve" are all based on ovarian response, not reserve. In this chapter, we will use "ovarian response" to refer to the state of ovarian function. As described below, many measures of ovarian response in women with FXPOI are consistent with significantly impaired ovarian function. However, we emphasize that FXPOI is not equivalent to an early natural menopause (Nelson et al. 2005). Menopause is defined as the permanent cessation of menses, and with this, the end of fertility. Women sometimes conceive naturally after getting the diagnosis of FXPOI.

As noted previously, POI is a more general term that is now used to encompass diagnostic conditions including what in the past has been referred to as premature menopause or POF, hypergonadotropic hypogonadism, and ovarian dysgenesis. Overall, this term describes impaired ovarian function on a continuum, rather than a specific endpoint (Welt 2008). The diagnosis and experience of POI is difficult as it can be transient and progressive (Nelson 2009). By the age of 20 years, POI affects about 1/10,000 women and 1/100 by the age of 40 years (Coulam et al. 1986; Cramer and Xu 1996; Goswami and Conway 2005; Kim et al. 1997). These estimates appear to differ by ethnic/racial group, indicating modifying factors (Luborsky et al. 2003). Both genetic and environmental causes of POI are known and have been extensively reviewed (e.g., Santoro, 2003; Cox and Liu 2014); however, the cause of POI in most women remains unknown.

The diagnosis of POI depends on the clinical picture (at least 4 months of oligo/amenorrhea) as well as the hormonal profile showing increased levels of FSH. Women with POI often go many years before a correct diagnosis is made. In fact, women with POI may have clinical symptoms wax and wane with periods of regular menses and amenorrhea further masking the underlying dysfunction. Natural menopause occurring after age 45 is often accompanied by hot flashes, night sweats, sleep problems, mood changes, and vaginal dryness (National Institute of Health 2005). POI occurs earlier in life and can be associated with residual ovarian function including return of menses, ovulation, and possible pregnancy (Hubayter et al. 2010; Nelson et al. 1994; Van Kasteren and Schoemaker 1999). The full experience of symptoms for natural age at menopause is becoming well recognized; however

for women with POI, more work needs to be done. Studies suggest that the unexpected and, sometimes intractable, infertility at a young age, along with early and prolonged exposure to estrogen deficiency can be traumatic and can exacerbate typical menopause-related symptoms. Indeed, Allshouse et al. (2015) have shown that menopause symptoms in women with POI are not adequately captured by the typical menopause symptom checklist. Their symptoms do not appear to diminish over time in contrast to women going through natural age at menopause. For example, in their sample, women with POI experienced depression prior to their diagnosis and hypothyroidism appeared to be more frequent compared with aged-norms.

Three potential mechanisms can underlie POI. These include: (1) a congenital decrease in primordial follicles, (2) accelerated follicular atresia, and (3) impaired ovarian follicle function. The various causes of POI are sometimes categorized as genetic, autoimmune, metabolic dysfunction, infectious, and iatrogenic; however, these groups clearly overlap as some are descriptive and some are etiologic based. Irrespective, FXPOI is a leading cause of the genetic forms of POI.

FXPOI: Clinical Description

The primary FXPOI-related trait that has been measured among women who carry the premutation is age at menopause, defined as cessation of menses for at least 1 year. As with all epidemiological studies of menopause, self-reported age at menopause is problematic. Pinpointing an event that has a long transition period and is somewhat ambiguous depending on symptoms is not easy. This is particularly true for cross-sectional surveys that involve recall. Moreover, many women are prescribed hormone medication as soon as cycle traits become variable or symptoms of menopause occur. Some women on hormone treatment may have had the ability to continue cycling naturally and some may not; hormone treatment masks this distinction. This, of course, complicates the ability to define a specific age at menopause, but if measured using the same definition within or among studies, significant patterns emerge among women who do and do not carry the premutation.

Increased Rate of POI Associated with the FMR1 *Premutation*

Schwartz et al. (1994) were the first to provide preliminary evidence for a premutation-associated reproductive disorder after the *FMR1* mutation was identified. Subsequently, a collaborative, multisite study was conducted to specifically confirm that women with the premutation, not the full mutation, were at risk for POI (Allingham-Hawkins et al. 1999). Although definitions of premutation allele sizes differed as well as the criteria for POI along with study protocols, the estimates of prevalence of POI, here defined as cessation of menses for at least 1 year prior to

age 40, among women who carry the premutation in subsequent studies all exceeded the expected 1 % in the general population (Allen et al. 2007; Coffey et al. 2008; Hundscheid et al. 2003a; Machado-Ferreira et al. 2004; Mallolas et al. 2001; Murray et al. 2000; Rodriguez-Revenga et al. 2009b; Sullivan et al. 2005; Uzielli et al. 1999a; Vianna-Morgante et al. 1999b; Wheeler et al. 2014). Based on a review of studies that interviewed women with the premutation at age 40 and older, the best estimate of the penetrance of POI was 15.5 % (70/451) (95 % CI: 12.3–19.2 %) and women without the premutation was 1.7 % (6/359) (95 % CI: 0.6–3.6 %). The estimate of early menopause, or menopause prior to the age of 45 years, was 20.0 % (47/235) (95 % CI: 15.1–25.7 %) and 4.0 % (6/149) (95 % CI: 1.5–8.6 %) among women with and without the premutation, respectively (Sherman et al. 2007). In a more recent study of 88 women with the premutation who reported experiencing menopause and were mothers of children with FXS, 35 % reported POI but only 18 % had been medically diagnosed with FXPOI (Mailick et al. 2014).

Defining a POI phenotype during adolescence is difficult due to the high variability in menstrual cycle characteristics that occur naturally during this reproductive stage (Chiazze et al. 1968; Treloar et al. 1967; Vollman 1977), in addition to the reduced ability to assess fertility. Combining data from two studies that obtained self-reported cessation of menses for at least 1 year prior to age 40 (Allingham-Hawkins et al. 1999; Uzielli et al. 1999b), 3 of 106 (~3 %) women with the premutation enrolled at ages 18–29 had already experienced POI. Combining data from two additional studies (Murray et al. 2000; Vianna-Morgante et al. 1999a), 7 of 217 (~3 %) women with the premutation reported the onset of menopause at or before age 29 and 3 of 217 (~1.4 %) reported menopause prior to age 18. These estimates among women with the premutation suggest a significant increase in risk compared with that in the general population, in which the incidence of menopause between ages 15 and 29 is estimated to be only 1 in 1000 (Goswami and Conway 2005).

Decreased Age at Menopause

An important question is to ask whether only a subset of women with the premutation are affected with FXPOI (incomplete penetrance) or whether the age at menopause distribution is decreased among all women with the premutation (variable expressivity). Although these alternatives are not mutually exclusive, they may provide insights toward mechanism and treatment. Previous studies estimated the age at menopause among women with the premutation using survival models. Sullivan et al. (2005) incorporated hormone use into their age at menopause definitions as follows: (1) age at her last menses irrespective of hormone use; (2) age at her last menses, but if she had started hormone medication prior to that event, she was censored (or dropped from the analysis) at the age she started hormone medication; or (3) age at last menses or age at the start of hormone medication. They found that the mean age at menopause among all women with the premutation given the

definitions above was 47.5 ± 0.7, 47.7 ± 0.8, and 46.6 ± 0.7 and among women without the premutation 52.2 ± 0.6, 53.1 ± 0.7, and 51.5 ± 0.6, respectively. These results show a consistent 5-year reduction in the age at menopause between women with and without the premutation, irrespective of the definition. Murray et al. (2000) found similar results using the more conservative definition of age at menopause, censoring women when they began hormone medication (definition #2 as defined above) and using survival analyses found similar results: 47.9 years ($SE_{mean} = 0.9$) among women with the premutation compared with 53.0 ($SE_{mean} = 0.8$) in women without the premutation. In a population-based sample of older adults in Wisconsin, 20 premutation carrier women had an average age at menopause (defined as last day of menstrual period) of 48.1 years compared with 50.8 years among women without the premutation ($n = 1893$) (Seltzer et al. 2012). This smaller difference may be due to the average repeat size identified among women with the premutation in this screened sample (see below).

Allen et al. (2014) tested the hypothesis that there is a subset of women who are at risk for ovarian dysfunction (i.e., incomplete penetrance). To do this, they simply examined the age at menopause distribution among women with the premutation, including and excluding those who reported cessation of menses for at least 1 year prior to age 40. They used the first definition described above (i.e., age at her last menses irrespective of hormone use). They considered this to provide a conservative estimate, as hormone use during transition may mask menopause and thus, lead a woman to report a later age at menopause. To test whether the differences in mean menopause age between women with and without the premutation were statistically significant, they estimated hazard ratios using a Cox proportional hazard model, adjusting for age at interview, racial/ethnic group, body mass index, and ever having smoked. For each premutation repeat group (55–79 repeats, 80–99 repeats and 100–200 repeats), the hazard ratio was significantly increased, indicating a higher risk for reaching menopause earlier among women with the premutation compared with women without the premutation, irrespective of the inclusion or exclusion of women who reported having FXPOI. This suggests that the majority of women with the premutation, not just a subset, experience ovarian insufficiency earlier than those without the premutation. This idea is supported by the results of Wheeler et al. (2014) who compared women with the premutation who did or did not have a diagnosis of FXPOI using a self-reported survey. They found that about 20 % of women without FXPOI also reported irregular or absent periods (18.4 %), early menopause (21.4 %), and 12.6 % also had difficulty getting pregnant.

Altered Hormonal Profile Among Women with Menstrual Cycles

There are many biomarkers that are used to indirectly measure ovarian response. These include the pituitary hormones (i.e., follicle stimulating hormone (FSH) and luteinizing hormone (LH)) and the ovarian hormones (i.e., estradiol (E2),

progesterone (P4), inhibin B, inhibin A, and anti-müllerian hormone (AMH)). FSH, a gonadotropin hormone, is the most established measure of ovarian response. AMH, also called müllerian-inhibiting substance (MIS), is a promising biomarker as many studies have reported a strong association between AMH levels and surrogate measures of ovarian response (including chronological age, antral follicle counts, time to menopausal transition and response to IVF) (e.g., De Vet et al. 2002; Fanchin et al. 2003; Seifer et al. 2002; Tremellen et al. 2005; Van Rooij et al. 2004). Also, several studies have found AMH to be more sensitive than the other commonly used markers (FSH, inhibin B, E2) (e.g., Fanchin et al. 2003; Hazout et al. 2004).

The most comprehensive study of the hormonal profile of women with the premutation was conducted by Welt et al. (2004). They hypothesized that women with the premutation with menstrual cycles would show hormonal changes characteristic of early age-related decline in ovarian function. They identified 11 women who carried the premutation who were still cycling, ages 24–41, and drew daily blood samples across one menstrual cycle. LH, FSH, E2, P4, inhibin A, and inhibin B levels were compared with levels in 22 age-matched, women without the premutation who were still cycling. They found that FSH was elevated across the follicular and luteal phases in women with the premutation compared to controls. Inhibin B in the follicular phase and inhibin A and progesterone in the luteal phase were all decreased in women with the premutation compared to controls. There was no difference in E2 or LH between groups. Thus, despite regular ovulatory cycles, FSH was increased in women with the premutation compared to controls and was accompanied by decreased inhibin B in the follicular phase and inhibin A and P4 in the luteal phase. These data are consistent with the hypothesis that women with the premutation show early age-related decline in ovarian function despite regular menstrual cycles. This profile may result from decreased follicle number and/or function, as reflected by lower inhibin B, inhibin A, and P4 levels.

In addition to this comprehensive study of a small number of women who carry the premutation, other studies have measured single serum FSH measurement taken during the early follicular phase of the cycle (day 2–5) among large study samples. This design is not ideal, as a woman's hormone profile can fluctuate significantly from month to month, particularly during the transition into menopause. Nevertheless, it allows a relatively noninvasive assessment of ovarian response and valid comparisons of means from large groups of women. Based on this design, the mean level of FSH among women who carried the premutation and were still cycling was significantly higher than that of noncarriers and of full mutation relative controls (Hundscheid et al. 2001; Murray et al. 1999) (adjusting for age and familial effects in the study of Murray et al. 1999 and adjusting for age, familial effects, smoking behavior, and use of oral contraceptives in the study of Hundscheid et al. 2001). In the study of Sullivan et al. (2005), they found increased levels of FSH only among women with the premutation aged 30–39 compared to women without the premutation. They did not find this same increase among women with the premutation in the younger or older age groups. They suggested that the lack of a difference among the older group (40–50 years) was due to a selection bias: those with POI (i.e., those who were not cycling) were not included in the study.

Rohr et al. (2008) measured both AMH and FSH in a cross-sectional study of women between ages 18 and 50 years. They found that serum AMH was reduced compared with women who do not carry the premutation, even at early ages (18–30 years). In contrast, elevated FSH indicative of early impaired ovarian response was only evident in older women who carried the premutation (31–40 years). These data suggested that AMH may be a better marker than FSH in identifying early stages of impaired ovarian response.

In a larger sample of 240 women ascertained through families with FXS ($n = 127$ women who carried the premutation and $n = 113$ women without the premutation; this sample includes that of Rohr et al. 2008), Spath et al. (2011a) found that women who carried the premutation showed reduced levels of AMH compared to women without the premutation at all ages. For all women, AMH was found to decrease by 10 % per year. The added effect of carrying a premutation decreased AMH levels by 54 %. They also modeled the decline in AMH levels and, using the subset of longitudinal samples ($n = 41$), found that it may be possible to create a standardized AMH using carrier status and age to potentially serve as a predictor for FXPOI.

Altered Menstrual Traits

Alteration of menstrual cycle traits can be the first indicator of POI and can provide insight into underlying pathology; thus, it is important to characterize these traits among women with the premutation. With respect to age at menarche, girls with the premutation appear to begin menses at the same time as girls without the premutation (Allen et al. 2007; Burgess et al. 1996; Hundscheid et al. 2003a). With respect to menstrual cycle characteristics, Schwartz et al. (1994) were the first to provide evidence that women with the premutation reported irregular menses more often than those without the premutation. Welt et al. (2004) showed that the length of the total cycle and of the follicular phase were significantly decreased in women with the premutation compared to their controls, whereas luteal phase length was similar. Subsequently, Allen et al. (2007) found that women with the premutation, and most significantly those with 80–100 repeats premutation alleles, reported shorter, irregular length and skipped cycles more often than women without the premutation. They found no difference in menstrual cycle bleeding length by carrier status. In the survey of Wheeler et al. (2014) women with FXPOI reported high rates of irregular or absent periods, early menopause, and difficulty getting pregnant at the time of the survey. The presence of these and of menstrual cycle symptoms were, of course, dependent on the diagnosis at the time the survey was taken.

Pre-, Peri-, and Postpartum Experiences

Kallinen et al. (2000) studied singleton pregnancies of 63 women with the *FMR1* expansions (55 with the premutation and 8 with the full mutation). Overall, they found that the pregnancy outcomes were favorable. The only

complication was that women who carried the mutation experienced more bleeding late in pregnancy than did the reference group. In the study of Wheeler et al. (2014), rates of preeclampsia or high blood pressure during at least one pregnancy were found to be higher among women with the premutation compared with the general population: about 16 % and 13 % of women with the premutation, with and without a diagnosis of FXPOI, respectively, reported these findings. Although rates did not differ among those with and without a diagnosis of FXPOI, this clinically significant medical condition should be explored further to determine the underlying cause of the increased rates. Similar to Kallinen et al., no other experiences with pregnancy, birth, or labor outcomes were different among women with the premutation compared with that expected in the general population.

Rates of Miscarriages Not Increased

In addition to hormonal fluctuations and changes in menstrual cycle characteristics, the age-related decline in ovarian function in women is associated with increased pregnancy loss resulting from aneuploidy. This age-related increase in aneuploidy results from an increased rate of chromosome nondisjunction in the ovary of older women (for review, see Sherman et al. 2013). Among women with the premutation, there is no evidence of an increased rate of miscarriages or aneuploidy (Allen et al. 2007; Hundscheid et al. 2003a; Murray et al. 2000).

Increased Rates of Twinning

Dizygotic twinning (DZ) is associated with advanced maternal age, ethnicity/race, and genetic factors. The association with maternal age is thought to be due to the elevated FSH levels and reduced ovarian feedback associated with diminished ovarian response, leading to an increased chance of multiple ovulations (reviewed in Lambalk et al. 1998). There have been conflicting reports of increased twinning among women who carry the premutation compared with women without the premutation (Allen et al. 2007; Fryns 1986; Hundscheid et al. 2003b; Murray et al. 2000; Sherman et al. 1996; Turner et al. 1994; Vianna-Morgante 1999). These variable results are probably due to the age and repeat-size characteristics of the study samples. For example, Allen et al. (2007) found increased twinning among only women with mid-range premutation repeat sizes who have the highest risk for FXPOI (see below), not among low and high premutation repeat groups.

Infertility and Subfertility

Perhaps the most significant clinical manifestation of FXPOI is infertility or subfertility early in reproductive life. The most comprehensive study to estimate the frequency of fertility problems is that of Wheeler et al. (2014). They found that 46.6 % of women with FXPOI ($n=73$) had difficulty getting pregnant, a significantly increased rate over those women with a premutation without a diagnosis of FXPOI ($n=365$). However, among those women without FXPOI, about 12.6 % reported difficulty getting pregnant. They also asked about reproductive assistance before and after they received a diagnosis of having a premutation. They found that all women with a premutation reported an increased frequency for fertility assistance, irrespective of their diagnosis of POI. Among women with and without a diagnosis of FXPOI, 31.1 and 8.5 % used some form of reproductive assistance prior to knowing their carrier status. Fertility drugs (e.g., Clomid) were most commonly used prior to carrier status diagnosis.

Early Symptoms of FXPOI

Few studies have assessed early symptoms of FXPOI. These are expected to be similar to those experienced by women transitioning into menopause, such as hot flashes (flushes), night sweats, irritability, poor concentration, depression, decreased interest in sex, and vaginal dryness. These symptoms may be particularly difficult to cope with for a young woman who is not anticipating such menopause-related symptoms early in life. In a descriptive assessment of 88 women with the premutation who had a child with FXS, the majority of women reported having at least some of these menopause-related symptoms (Mailick et al. 2014). The most common symptom was hot flushes/flashes (30.7 % experiencing this symptom “a lot”). In addition, night sweats (26.1 % experienced this symptom “a lot”), sleep disturbance (22.7 %), and depression (19.3 %) were reported.

Wheeler et al. (2014) found that headaches, menstrual symptoms, and hot flashes were reported by women with the premutation, irrespective of their diagnosis of FXPOI. The frequency of reporting tended to decrease in the older age groups, while joint pain, for example, tended to be reported at higher rates by older women. There is some evidence that experiencing such symptoms or reporting of such symptoms may be increased among women with increased stress. For example, Smith et al. (2012) surveyed mothers of adolescents and adults with FXS ($n=112$), mothers of adolescents and adults with autism spectrum disorders (ASD, $n=96$) and compared them to a nationally representative sample of mothers with similarly aged children without disabilities ($n=230$). They found that both groups of women with children who had disabilities reported a significantly increased frequency of hot flashes/flushes compared to controls.

Other Associated Disorders

Women with the premutation are more likely to experience estrogen deficiency at an earlier age than women in the general population. As such, the premutation becomes a biomarker for a population of women who are at risk of early estrogen deficiency-associated morbidity and mortality. Hundscheid et al. (2003a, b) were the first to investigate medical conditions associated with estrogen deficiency among 152 women with the premutation and 112 controls (women with the full mutation or women with a normal *FMR1* genotype), all ascertained from families with FXS. They asked about the following medical conditions: hypertension, hypercholesterolemia, cardiovascular disease, thrombosis, reduction of bone mineral density, subfertility, uterus extirpation, rheumatoid arthritis, type 2 diabetes, hyperthyroidism, hypothyroidism, and reproductive-associated cancers (breast, ovarian, endometrial). The only statistically significant difference between groups was the increase in bone loss among women with the premutation. Among the women with the premutation who reported bone loss, all went through menopause before the age of 44 years. Allen et al. (2007) also found an increase in self-reported osteoporosis, but only among the highest risk group for FXPOI (those with 80–100 repeats). Other health conditions specifically reported (autoimmune disorders, lupus, diabetes, Graves disease, breast cancer, ovarian cancer, hysterectomy) were not increased among women with the premutation. One limitation of both studies was that the study population was relatively young for these later-onset complications.

Several studies compared health conditions among women with and without indications of POI (e.g., irregular cycles) or a diagnosis of FXPOI. Among 325 women with the premutation, Hunter et al. (2010) found that women who were experiencing irregular cycles at the time of their evaluation reported higher rates of thyroid problems and depression/anxiety compared with those with no indication of ovarian dysfunction. Kenna et al. (2013) studied a small sample of 46 mothers who had at least one child with FXS. They found that women with the premutation had an earlier mean age of menopause (mean age = 45.6 years) and a high rate (76 %) of lifetime depressive or anxiety history, with 43 % of the reporting a comorbid history of both diagnoses. However, they found no evidence that their psychiatric history was related to their ovarian dysfunction.

In the largest study to date, Wheeler et al. (2014) separated women by their diagnosis of FXPOI ($n = 75$ with and 365 without FXPOI) and compared their self-reported diagnostic history of seven medical conditions, some of which are associated with estrogen deficiency: thyroid disease, hypertension, autoimmune disease, heart disease, gastrointestinal (GI) issues, seizures, and diabetes. Although they found high reporting rates, especially thyroid (24 and 19 % among those with and without FXPOI) and GI problems (32 and 23 % among those with and without FXPOI), there was no difference based on their FXPOI diagnosis. Increasing age was a significant predictor of the frequency of most of these conditions. Winarni et al. (2012) focused on the risk of immune-mediated disorders (IMDs) among women with the premutation using a survey. Among women who were 40 years or

more, there were increased rates of IMD among the 41 women with the premutation with a diagnosis of FXPOI (66 %) compared with 147 women with the premutation without FXPOI (46 %) and compared with 50 controls (34 %). Among women with the premutation, the most commonly reported IMD was autoimmune thyroid disorder (24.4 %).

Wheeler et al. (2014) also asked about five psychological or educational (depression, anxiety, ADHD, learning disabilities, speech/language disorder) diagnoses. As in Kenna et al. (2013), depression and anxiety were both reported at high rates: about 40 % of women with and without FXPOI reported a history of these diagnoses, but the presence of these conditions was not associated with FXPOI. Lastly, Wheeler et al. (2014) probed women about specific physical symptoms that have been reported in the literature (see Chap. 13 for details). These included questions on symptoms such as headache, fatigue, joint pain, and menstrual cycle problems experienced over the previous 30 days. They found that fatigue was the most common daily symptom endorsed: 49 and 35 % of women with and without FXPOI reported fatigue. Women with FXPOI were significantly more likely to report muscle weakness, dizziness, and nausea compared with women who were not diagnosed with FXPOI.

Risk Factors of FXPOI

Not all women with the premutation experience FXPOI; some go through menopause at ages similar to women without the premutation. Four factors have been examined to try to explain the incomplete penetrance of POI among women with the premutation: (1) CGG repeat length, (2) skewed X-chromosome inactivation (XCI), (3) smoking, and (4) background genes. With respect to *FMR1* repeat length, there is a strong nonlinear association of repeat size with severity of symptoms of FXPOI. For example, women with mid-range premutation repeats (~80–100 repeats) have the highest risk for FXPOI. Women who carry both smaller or larger premutation repeat alleles also have an increased risk of FXPOI compared to the general population, but not to the same extent as women with the mid-range premutation repeat length (Allen et al. 2007; Ennis et al. 2006; Mailick et al. 2014; Spath et al. 2011b; Sullivan et al. 2005; Tejada et al. 2008). Women with mid-range repeats have the lowest mean age at menopause compared to the other groups, an indication of increased severity of FXPOI. Using survival analysis, the unadjusted mean age at menopause for women without the premutation and for women in the three premutation categories, low (55–79 repeats), mid (80–100 repeats) and high (100–200 repeats), were 52.3 + 0.5, 48.5 + 0.7, 44.9 + 0.6, and 47.5 + 1.2, respectively (Allen et al. 2007). Similarly, symptoms of FXPOI were most frequently reported by women with the mid-range premutation. Such women had an increased risk for altered cycle traits (shortened cycle length, irregular cycles, and skipped cycles), subfertility, and dizygotic twinning. This increase in symptoms is not unexpected given the 7-year reduction in age at menopause among women with the mid-range

premutation repeat length (Allen et al. 2007). These definitions of low, mid, and high premutation alleles do not have specific biological meaning. Indeed, in an analysis that did not predefine repeat size groups, women with alleles between about 65 and 90 repeats had the highest risk for FXPOI (as defined by a hazard ratio and the 95 % confidence intervals exceeding 2) (Spath et al. 2011b). Clearly, more work is needed to define the repeat size alleles that impose the highest risk and why.

As the *FMR1* mutations are located on the X chromosome, it is important to consider skewed XCI is an important modifier of the risk for FXPOI. No study has found evidence for skewed XCI based on samples from fresh blood among women with the premutation (Bione et al. 2006; Mailick et al. 2014; Murray et al. 2000; Rodriguez-Revenga et al. 2009a; Spath et al. 2010; Sullivan et al. 2005; Tejada et al. 2008). Assuming that XCI in blood can be used as a proxy for the correct target tissue, one possible explanation for this observation is that the toxic effect of the premutation acts during a stage in development when both X chromosomes are active.

Lifestyle factors have been investigated as modifiable factors that may affect natural age at menopause (e.g., Sapre and Thakur 2014; Schoenaker et al. 2014). Smoking, an important modifiable risk factor, is known to reduce age at menopause. With respect to women with the premutation, smoking was also found to reduce age at menopause/FXPOI (Allen et al. 2007; Spath et al. 2011b). Importantly, the effect size of this modifiable factor appears to be similar in women with and without the premutation.

The influence of genetic variants on the onset of natural menopause is now well established (e.g., Chen et al. 2014; He et al. 2009; Shen et al. 2013; Stolk et al. 2012). Many of these variants are also found to influence age at menopause among those with early menopause or POI (e.g., Perry et al. 2013; Qin et al. 2012). An important question is whether these genetic variants also play a role in FXPOI (i.e., are such variants also important when in the presence of a single mutation of large effect size on ovarian function?). There is both indirect and preliminary direct evidence that background genes are important in defining the age of onset of menopause/FXPOI in women with a premutation.

The indirect evidence comes from two studies. Hunter et al. (2008) used a random-effects Cox proportional hazards model to analyze age at menopause on 680 women (carriers and noncarriers) drawn from 225 families with FXS and 321 women from 219 families drawn from the general population. They found a statistically significant residual additive genetic effect after adjustment for repeat length and other covariates. The study of Spath et al. (2011b) took a different approach using mean age at menopause of first-degree relatives with the same mutation status as a predictor variable for age at menopause along with smoking and repeat length. Their sample included data sets from the USA and from the Netherlands consisting of 1068 women. They found that the mean age at menopause of first-degree relatives was a statistically significant predictor of age at menopause, adjusting for repeat size and smoking status, only for women without the premutation, not for

women with a premutation. This may be due to the lower power to detect such background gene effects in the presence of the major gene effect of the premutation or to the limited information in the definition of the predictor variable.

Direct evidence for the role of genes modifying the penetrance of FXPOI is mounting. First, in a preliminary study, five common single nucleotide polymorphisms (SNPs) known to influence age at menopause and age at POI (Perry et al. 2013; Stolk et al. 2012) were examined among 72 women with the premutation and known age at menopause (Allen et al. 2014). Because of the small sample size, only one primary statistical test that combined the effect of all five SNPs was conducted. They calculated "Total SNP Risk," or the sum of the number of risk alleles among the five SNPs, weighted by the effect size per allele. They used linear regression with age at menopause as the outcome variable and repeat size and Total SNP Risk as predictor variables. The Total SNP Risk was significantly associated with age at menopause after adjusting for effect of the premutation repeat.

Others have taken a different approach to identify misregulated gene expression among carriers of the premutation. Mateu-Huertas et al. (2014) used gene expression profiling among men with and without the premutation and identified one differentially expressed gene, *Early at Menopause 1* (*EAP1*), as a potential candidate for modifying the severity of FXPOI. This gene is a component in the hypothalamic control for the initiation of estrus in rodents and puberty and menstrual cycles in nonhuman primates (Heger et al. 2007; Lomniczi et al. 2012). To follow this lead, expression levels of *EAP1* were compared among the following women: 12 women with a premutation and FXPOI, 13 women with a premutation without FXPOI, eight women without the premutation, and four women with POI who did not have a premutation. *EAP1* was significantly downregulated in women with a premutation and FXPOI compared with those without FXPOI. In addition, *EAP1* was also significantly downregulated in women with POI without a premutation compared with controls. Thus, decreased *EAP1* levels may contribute to the ovarian dysfunction in women with FXPOI.

Alvarez-Mora et al. (2015) used whole genome expression arrays to study six women with the premutation and FXPOI and six premutation women without FXPOI along with four women without the premutation or POI. Although they did not find any gene with a significant differential gene expression, they noted pathways that showed significant alterations, primarily downregulation, of associated genes in women with FXPOI. Gene annotation and gene-set enrichment methods of the gene expression profiles suggested that FXPOI may be due to generalized deregulation of key signaling pathways involved in oocyte maturation.

As pointed out in both gene expression profiling studies, the limitation of this approach is based on the use of blood instead of the target tissue involved in FXPOI. Nonetheless, these studies provide clues that can complement results from model systems to identify important pathways on which to concentrate efforts to find therapeutic targets (see Chap. 11).

Management of FXPOI

FXPOI is one of the few conditions of POI for which a known risk factor exists, namely carrying the *FMR1* premutation. This is a significant advantage, as there are ways to help manage the potential consequences of the condition prior to onset of symptoms. Layered onto this actionable situation is the complication of risk for having a child with significant behavioral problems and intellectual disability, i.e., having a child with FXS. Without trying to minimize this complex situation, here we will only outline management of FXPOI, acknowledging that the diagnosis of carrying the premutation brings challenges to a woman that are far beyond this scope of the following discussion.

Diagnosis

The difficulty for a woman getting the diagnosis of POI has been discussed thoughtfully and emphasizes the need to have health professionals spend the needed time to deliver the information to a woman in an appropriate setting, as this news may lead to shock, anxiety, depression, and the stigmatization of infertility (Sterling and Nelson 2011). For women in this situation, the diagnosis of POI typically comes first, followed by further work-up for the potential cause. The American College of Obstetricians and Gynecologists recommends that all women presenting with POI should be tested for the premutation, regardless of their family history (ACOG 2010). Pre-test counseling for FXPOI to inform a woman about the potential of a diagnosis of FXPOI and other associated risks could be helpful; however, as only about 3 % of women with POI will be found to carry the premutation, sometimes pre-test counseling may increase anxiety.

For a woman who carries the premutation, her carrier status is most often identified through the presence of symptoms of FXS in her offspring or another family member. At that time, she is seeking information about FXS and how to care for her child. She is most often in the hands of a pediatrician, clinical geneticist, or genetic counselor at this point. She may be seeking information about her risk for having a child with FXS, but she is most likely not seeking information about POI. Clinicians need to be aware that the discussion about the woman's risk of FXPOI in this scenario may not be heard (Espinel et al. in press). Thus, follow-up with a reproductive endocrinologist or obstetrician/gynecologist is most likely necessary.

In either diagnostic situation, women will need significant health professional and social support assistance (Sterling and Nelson 2011). Women with a premutation may perceive or experience stigmatization resulting from carrying a gene that may lead to offspring with intellectual disability and possibly autism, to premutation-associated disorders of FXPOI and FXTAS, and to the specific outcome of infertility. Comments from others that infertility is beneficial to one who has a risk for passing the mutation for FXS are damaging. Stigma is associated with anxiety and

depression (Davis et al. 2010; Slade et al. 2007). Health professionals can draw from the rich literature on ways to help reduce perceived loss of social support and low self-esteem that often come with stigmatization (Sterling and Nelson 2011).

Women with premutation or FXPOI may experience grief or loss or disruption of their life plan when they receive their diagnosis. A woman's pursuit for childbearing and for children free from intellectual hardships is central to many. Sterling and Nelson discuss possible ways to help navigate through this transition towards a new life plan for women with a diagnosis of POI. These suggested ways to help build on a woman's inherent positive psychological resources to redefine life goals are important and, again, must be considered in the context of both FXPOI and risk for FXS in offspring.

Management Prior to Onset of Symptoms of FXPOI

For FXPOI, self-management can begin prior to onset of symptoms. As soon as a woman is identified as carrying the premutation, she should become aware of possible medical concerns. As not all women with the premutation will experience FXPOI, it is important to identify biomarkers that can be monitored to identify those at high risk for a shortened reproductive life span and onset of estrogen deficiency. After establishing supportive evidence, biomarkers such as cycle variability, AMH, and FSH could be measured when a woman is first diagnosed as carrying the premutation and monitored on a regular basis. As noted above, AMH may be a better marker for early stages of POI compared with FSH. At this point, we know very little about the occult stages of FXPOI in adolescents. Based on the fact that age at menarche does not seem to be affected in FXPOI and that cycles can be highly variable, as well as measures of AMH during adolescents in all women, perhaps monitoring should begin around age 18 years. Although the predictive value of these biomarkers for FXPOI has not been studied longitudinally, they may be helpful in identifying which women with the premutation are at higher risk for FXPOI and may need more counseling from a reproductive endocrinologist about monitoring, potential fertility preservation options, and hormone therapy if cycles become irregular. It is important to stress, however, that having diminished ovarian response as evidenced by abnormally low AMH or abnormally high FSH does not necessarily equate with infertility or impending secondary amenorrhea.

Management at Onset of Symptoms of FXPOI

POI leads to early estrogen deficiency. As such, hormone treatment is important to the health of girls and young women experiencing FXPOI. Most experts agree that estrogen and progestin replacement is indicated for women with POI and that

replacement should continue until they reach the typical age at menopause (Board of the International Menopause Society et al. 2007; Nelson 2009; Practice Committee of the American Society for Reproductive Medicine 2004; Rebar 2009; Welt 2008).

As noted above, loss of bone density is reported more often among women with the premutation. This is a consequence of the early estrogen deficiency, although a direct effect of the premutation on bone turnover has not been studied. A bone density study is indicated once a woman has confirmed FXPOI with associated oligo/amenorrhea. To maintain wellness once symptoms of FXPOI occur (e.g., cycle variability, altered hormone profile), women with a premutation should be aware of bone health maintenance, as outlined for perimenopausal women in the North American Menopause Society (North American Menopause Society 2006). This includes maintaining adequate vitamin D levels, supplementation of calcium intake, and establishing a regular mixed exercise program of loading exercise and resistance training. There is important new evidence from a controlled trial regarding hormone replacement therapy (HRT) for women with POI. The study compared bone mineral density over 3 years in a group of control women with normal ovarian function and a group of women with POI taking a physiologic HRT (100 μg/day estradiol patch, 10 mg per day oral medroxyprogesterone acetate for 12 days each month). At study entry, women with POI had significantly lower bone mineral density compared to controls. By study end, bone mineral density had increased markedly in women with POI to such a degree that it did not differ from bone density in the control group. Thus, the study showed that not only could this regimen of HRT reduce the rate at which women with POI lose bone mineral density, it could actually restore bone density to normal (Popat et al. 2014). This study sets the HRT standard by which to compare any other HRT regimens in women with POI. Evidence also suggests that this regimen promotes better bone health in women with POI than using an oral contraceptive for HRT. The oral contraceptive suppresses bone formation markers in these women, whereas transdermal estradiol significantly increases bone formation markers. Because of the uncertain effects of bisphosphonates on the fetus and their long skeletal half-life, these agents are not recommended in women who might conceive (Drake et al. 2008).

After recovering emotionally from being informed of the diagnosis of FXPOI, there is much to consider with respect to family planning. One important issue is to understand that some 5–10 % women with a diagnosis of POI can have spontaneous ovarian function and can conceive without medical intervention. Thus, for women who do not want to become pregnant, contraception must be considered. Because the effectiveness of oral contraception has not been evaluated among women with FXPOI, a barrier contraceptive method or intrauterine device should be considered.

For each woman with the premutation and potentially with FXPOI, her decision about family planning is highly personal. Options include making the decision not to have offspring, trying to conceive naturally, adoption, foster parenting, and medical intervention. The latter can include in vitro fertilization along with preimplantation genetic screening or egg or embryo donation. Chapter 13 provides more detail

about the various options with respect to family planning in the context of fragile X-associated disorders. The important point to make is that each woman needs to make her own choices on her own time schedule. Both social and medical support should be available if she needs it.

Future Studies and Conclusions

The underlying etiology of FXPOI is still unknown. At this point, there are established guidelines to help manage the symptoms of FXPOI, but it is important to continue the search for the underlying etiology. FXPOI is one of three established fragile X-associated disorders that are the consequence of the repeat expansion in the 5′ untranslated region of the *FMR1* gene. As noted above, FXPOI is limited to women who carry the premutation, not the full mutation. This suggests that reduction of FMRP, the protein that is not produced from full mutation alleles, is not the primary cause of FXPOI. Instead, some characteristic of the premutation allele is the culprit. There are important molecular consequences of the premutation: with increasing repeat length, there is increasing *FMR1* mRNA levels and decreasing FMRP levels (Allen et al. 2004; Garcia-Alegria et al. 2007; Kenneson et al. 2001; Peprah et al. 2010; Tassone et al. 2007). Many have postulated that *FMR1* mRNA toxicity may underlie FXPOI, as is the case for the other premutation-associated disorder, FXTAS. Chapter 11 reviews the evidence for the proposed mechanisms that may play a role in FXPOI. Two prevailing proposals include sequestration of important binding proteins or production of novel proteins with different homopolymeric or heteropolymeric amino acid tracts. Both of these outcomes are known to exist in FXTAS and are related to the unusual secondary structures that are formed as a result of the large repeat track in the premutation mRNA. Chapter 11 also describes the mouse models for FXPOI and shows evidence that, at least for these models, the premutation allele does not affect the primordial follicle pool. They also show that there is no specific block in follicular development or premature activation of follicles. Instead, data suggest that the problem may be related to abnormal granulosa cell proliferation. The importance of understanding the mechanism cannot be overstated. Once identified, possible interventions can be considered and well as possible biomarkers that indicate risk for a shortened reproductive life span.

In parallel, natural history studies of women with the premutation, starting as early as possible and continuing longitudinally, would be important to understand how early occult symptoms of FXPOI begin and to determine the comorbid associations of the disorder. These studies would help identify how best to manage outcomes of FXPOI in terms of timing and treatment regimes.

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Chapter 11
Model Systems for Understanding FXPOI

Karen Usdin, Renate K. Hukema, and Stephanie L. Sherman

Abstract Fragile X-associated primary ovarian insufficiency is a poorly understood cause of infertility and ovarian insufficiency. Studies to date in humans have been limited for the most part to studies of the epidemiology and natural history of the condition in women. However, related Repeat Expansion Diseases as well as cell and animal model systems are beginning to provide vital insights into the mechanisms that might be responsible for disease pathology.

Keywords Repeat expansion disorder • *FMR1* • Fragile X • Infertility • Ovarian insufficiency • Early menopause

Introduction

As discussed in previous chapters, Fragile X-associated primary ovarian insufficiency (FXPOI) is a member of the *FMR1*-associated disorders, a group of diseases arising from inheritance of an X-linked gene, *FMR1*, that has a greater than normal number of CGG/CCG-repeats in its 5′ untranslated region. These disorders belong to a larger group of genetic disorders known as the Repeat Expansion Diseases that all result from inheritance of an allele of a disease-specific gene with too many repeats. In the case of the *FMR1*-associated disorders, alleles with 55–200 CGG/CCG-repeats are known as premutation (PM) alleles and inheritance of such alleles confers risk of FXPOI as well as fragile X-associated tremor/ataxia syndrome

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(FXTAS), a neurodegenerative condition that is discussed in detail in Chaps. 1–9 of this book. Women who carry the PM are also at risk of having a child with fragile X syndrome (FXS), the most common heritable cause of intellectual disability. FXS results from the generation on maternal transmission of a full mutation (FM) allele that has >200 CGG/CCG-repeats (see Chonchaiya et al. 2009 for recent review).

Clinical Characteristics of FXPOI

The clinical features of FXPOI are described in more detail in the preceding chapter (Chap. 10). Briefly, a hallmark of women with FXPOI is experiencing at least 4 months of oligo/amenorrhea before the age of 40. They also have hypergonadotropic hypogonadism and display various occult indicators of ovarian dysfunction including decreased levels of anti-Müllerian hormone (AMH) and increased levels of follicle stimulating hormone (FSH) consistent with an early ovarian insufficiency phenotype (Welt et al. 2004). This spectrum of conditions is best referred to as primary ovarian insufficiency (POI). However, it is not known whether these changes are due to a smaller than normal original primordial follicle pool in these women or a more rapid than normal depletion of that pool. Whatever the basis of the diminished ovarian function at diagnosis, the most immediate and significant consequence is reduced fertility (Allen et al. 2007; Streuli et al. 2009). However, the premature loss of estrogen resulting from early ovarian insufficiency also has important clinical consequences including an earlier onset of osteoporosis and an increase in bone fractures (Gallagher 2007; Vega et al. 1994), impaired endothelial function (Kalantaridou et al. 2004), and an earlier onset of coronary heart disease (Atsma et al. 2006). In fact early menopause is associated with a higher mortality from both cardiac and noncardiac causes (e.g., Maclaran et al. 2010; Shuster et al. 2010). In addition, women with POI have more anxiety, depression, and psychological issues than women with normal ovarian function (Deeks et al. 2011; Rodriguez-Revenga et al. 2008).

About 20 % of women with the PM have cessation of menses prior to age 40. This corresponds to a 20-fold higher rate than is seen in the general population (for reviews, see Sherman 2000; Sullivan et al. 2011). However, the number of women with the PM who have occult or clinical signs of ovarian dysfunction while still cycling is unknown, as is the extent to which such indicators predict infertility or POI. This is a significant issue as at least 1/250 women carry the PM allele. There is a strong nonlinear association of the penetrance of POI, with ~70–100 repeats conferring the highest risk (Allen et al. 2007; Ennis et al. 2006; Mailick et al. 2014; Spath et al. 2011; Sullivan et al. 2005; Tejada et al. 2008). The basis of this nonlinear relationship is unknown, as is the reason for the incomplete penetrance of the disorder. Presumably there are one or more genetic and/or environmental modifiers of disease risk. For example, the age at menopause of women with the PM, and thus their risk of FXPOI, is influenced by their genetic background (Hunter et al. 2008;

Spath et al. 2011). In addition, smoking, which is a known risk factor for a reduced age at menopause in general, has also been shown to affect the risk of FXPOI (Allen et al. 2007; Spath et al. 2011).

Since the *FMR1* gene is located on the X chromosome, in principle skewed X inactivation (XCI) could affect the risk or severity of FXPOI. However, no evidence for an effect of skewed XCI has been found in fresh blood among women with the PM who have FXPOI (Bione et al. 2006; Mailick et al. 2014; Murray et al. 2000; Rodriguez-Revenga et al. 2009; Spath et al. 2010; Sullivan et al. 2005; Tejada et al. 2008). If XCI in blood is an appropriate proxy for the correct target tissue, this may point to the deleterious effect of the PM occurring in the oocyte since it is the only differentiated cell in which both X chromosomes are active.

The FMR1 Gene Product, FMRP, Is Highly Expressed in the Ovary but Is Unlikely to Contribute to FXPOI

The *FMR1* gene encodes an RNA-binding protein, FMRP. FMRP is a nucleocytoplasmic shuttling protein that transports a subset of transcripts to specific sites in the cytoplasm where it is then involved in the regulation of their translation (Laggerbauer et al. 2001). Current thinking favors a model for FMRP function via its interaction with components of the RNA interference (RNAi) pathway to downregulate translation of the mRNAs to which it binds (Caudy et al. 2002; Ishizuka et al. 2002; Jin et al. 2004). FMRP also plays a less well-understood chromatin-dependent role in the DNA damage response (Alpatov et al. 2014). The role of FMRP has been studied most intensively in the brain, since its absence is associated with intellectual disability. However, FMRP is also highly expressed in the granulosa cells and oocytes of humans (Rife et al. 2004), rodents (Bachner et al. 1993; Ferder et al. 2013; Hoffman et al. 2012; Takahashi et al. 2015) and the ovary of flies (Costa et al. 2005). In flies, the *FMR1* homolog, dFMR, modulates the proliferation of both male and female germ line stem cells. Female flies lacking dFMR show impaired fertility and produce fewer egg chambers than wild-type flies (Epstein et al. 2009; Yang et al. 2007, 2009). However, flies only have one member of the dFMR family whereas mammals have three, *FMR1*, and two autosomal homologues, FXR1 and FXR2. Thus how relevant the fly data are for our understanding of mammalian FMRP is unclear. In fact, in mice the absence of FMRP results in ovarian overgrowth and a larger number of ovarian follicles compared to wild-type animals (Ascano et al. 2012) suggesting that mammalian FMRP may not act in the same way.

An increasing repeat number is associated with a reduction in FMRP (Kenneson et al. 2001; Ludwig et al. 2014; Primerano et al. 2002) due to the poor translation of *FMR1* transcripts with large numbers of repeats (Feng et al. 1995). Given the ovarian phenotype seen in *Fmr1* null mice (Ascano et al. 2012), a decline in FMRP levels could, in principle, contribute to FXPOI. However, women with the FM make

much less FMRP than women with the PM because of repeat-mediated silencing of FM alleles. Yet they do not have ovarian dysfunction characteristic of FXPOI. Thus, FXPOI pathology is unlikely to result from a decline in the level of FMRP per se.

FXPOI Is Likely an RNA Gain-of-Function Disorder

Current thinking is that FXPOI pathology arises from some *FMR1* mRNA gain-of-function as has been suggested for FXTAS (Hagerman et al. 2001). However, whether the two disorders arise from the same basic problem is not known. Of interest in this regard is that women with FXPOI do not necessarily have FXTAS and vice versa. This does not necessarily mean that the pathological mechanisms are different. It may be that different modifying factors affect FXPOI and FXTAS penetrance. However, the intranuclear inclusions that are one of the most striking hallmarks of FXTAS have not been detected thus far in either granulosa cells or oocytes of PM women (Chang et al. 2011). It may be that inclusions do form in these cells but do not get big enough to be visualized microscopically before death of the oocyte occurs or that the inclusions are protective rather than pathological and thus not necessarily a good indicator of the cell types most at risk. It is also possible inclusion formation does not drive pathology or that the site of FXPOI pathology lies outside of the ovary in cells that do show inclusions. Curiously, inclusions have been seen in stromal cells of the PM ovary, a cell type in which FMRP is not highly expressed (Chang et al. 2011).

Lessons from Related Diseases and Model Systems

Many other Repeat Expansion Diseases, including FXTAS, have been more intensively studied than FXPOI both in affected humans and in different cell and animal models. However, there are reasons to think that insights from these disorders and their model systems may inform our understanding of FXPOI. Mouse models of FXPOI itself are also beginning to provide specific information as to the consequences of expression of the PM in the ovary. This chapter will review what insights into FXPOI we have gained thus far from the study of these different systems.

Lessons from the Other Repeat Expansion Diseases

A number of Repeat Expansion Diseases result from the presence of a large repeat tract in the 5′ UTR, in an intron, or in the 3′ UTR of the affected gene (reviewed in Ranum and Cooper 2006). Since the normal open reading frame of the gene is not affected, these disorders are all thought to arise from some toxic consequence of the

production of a transcript containing these repeats. Many of these repeats form stable RNA hairpins that have a number of potentially relevant biological consequences. For example, some of these hairpins are substrates for Dicer (Handa et al. 2003; Krol et al. 2007), a component of the RNA interference machinery that is involved in posttranscriptional gene regulation. Dicer generates small single stranded RNAs of ~21 nt and such repeat-containing RNAs have been seen in the brains of HD patients. These RNAs reduce the translation of other repeat-containing transcripts and are toxic to human cells (Banez-Coronel et al. 2012).

However, most current models for repeat pathology in these disorders are based on two other potentially deleterious consequences of repeat-containing hairpins (Fig. 11.1). The first model, the Protein Sequestration model (Fig. 11.1), is based on studies of myotonic dystrophy type 1 (DM1) and type 2 (DM2). These disorders are thought to arise in large part because the RNA hairpins produced from the affected genes are bound by and sequester proteins that are essential for normal cell functioning (Mankodi et al. 2001; Miller et al. 2000). Nuclear foci (inclusions) are seen in affected cells (Taneja et al. 1995) that contain both the repeat-containing transcript and members of MBNL (muscleblind-like) family of splicing factors

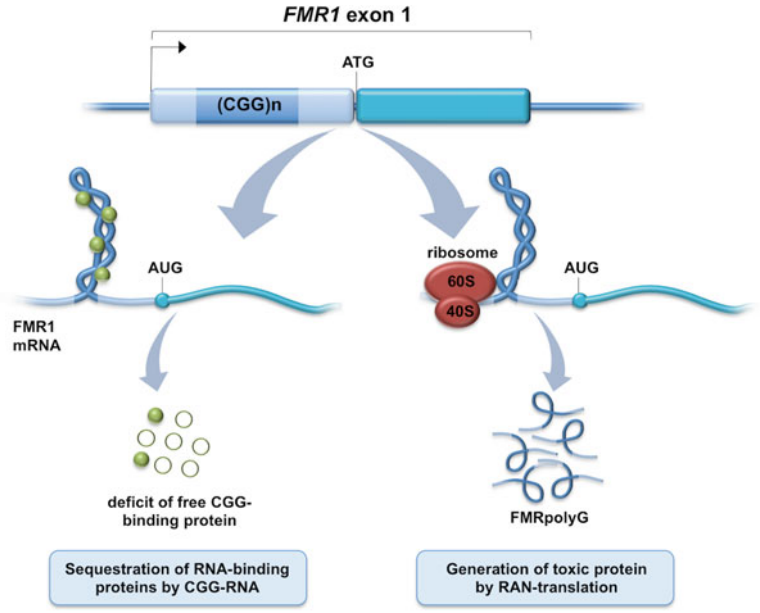

Fig. 11.1 Diagrammatic representation of the two major models for RNA-mediated repeat pathology in the Repeat Expansion Diseases using the FX PM as an example. The Protein Sequestration model is illustrated on the *left* hand side and the RAN translation model on the *right*. The CGG-hairpin formed in the PM transcript binds and sequesters proteins important for normal cell function. This results in a deficit of these proteins that affects cell viability. In the RAN translation model, the CGG-RNA hairpin promotes the initiation of translation at a near AUG-codon upstream of the repeat. Translation through the repeat results in the generation of a protein, FMRpolyG, that has long polyglycine tract (Todd et al. 2013)

(Mankodi et al. 2001; Miller et al. 2000). These proteins are known to bind to the RNA hairpins formed by disease repeats in vitro (Miller et al. 2000). Affected individuals show splicing abnormalities consistent with depletion of MBNL proteins and these abnormalities are thought to contribute to disease pathology. Disruption of the gene in which the repeat is located does not recapitulate disease pathology in mice, whereas disruption of members of the *Mbnl* gene family does (Lee et al. 2013), thus supporting the idea that pathology arises because of a deficiency of free MBNL proteins.

The second model (Fig. 11.1) for RNA hairpin-induced pathology involves repeat-associated non-AUG initiated translation (RAN translation), a form of translation that is initiated at an atypical start codon either upstream of or, in some cases, within the repeat (Cleary and Ranum 2013). How RAN translation is actually initiated is unknown. It may be that the RNA hairpins act in a manner analogous to that proposed for the stem-loop region responsible for initiation at non-AUG codons in some picornaviruses (Sasaki and Nakashima 2000). Whatever its molecular basis, the net effect of this translation is that one or more novel proteins are produced that include the products of translation through the repeat tract. Depending on the repeat involved and which non-AUG codon is used for initiation, this can result in the production of a variety of proteins with different repeated amino acid tracts. How these proteins exert pathology is not known, but they are frequently associated with the formation of large ubiquitinated intranuclear inclusions. It has been suggested that these proteins act in a manner analogous to the polyglutamine proteins that result from AUG-initiated translation through CAG-repeat tracts that occurs in some of the other Repeat Expansion Diseases (Cleary and Ranum 2014).

Lessons from FXTAS and FXTAS Model Systems

Expression of PM length CGG-repeats with, or without, the *FMR1* ORF is toxic to fly and mammalian cells (Handa et al. 2005; Hashem et al. 2009; Hukema et al. 2014; Jin et al. 2003; Todd et al. 2010). This would be consistent with the idea that the pathology associated with the PM is not related to the levels of FMRP per se but is related instead to the deleterious consequences of the production of a transcript with large numbers of CGG-repeats. Expression of CGG-RNA is also associated with the formation of intranuclear inclusions in a variety of cell types (Buijsen et al. 2014; Greco et al. 2002; Hunsaker et al. 2011; Iwahashi et al. 2006; Oh et al. 2015; Tassone et al. 2004; Willemsen et al. 2003). These inclusions are reminiscent of those seen in disorders that are thought to result from either protein sequestration or RAN translation. In addition, CGG-repeats form RNA hairpins that are similar to those formed by the repeats responsible for these disorders (Handa et al. 2003; Zumwalt et al. 2007) and evidence for both protein sequestration and RAN translation is seen in FXTAS patients as well as cell and animal models (as illustrated in Fig. 11.1).

A number of different proteins are sequestered either directly or indirectly by the CGG-RNA. These are discussed in more detail in Chaps. 6 and 8. Briefly, in fly models sequestered proteins include the multifunctional protein Pur α (Jin et al. 2007), the splicing factor and neuronal RNA transport protein, hnRNP A2/B1 (Sofola et al. 2007), the miRNA processing factor DGCR8 (Sellier et al. 2013), MBNL1 and the splicing factor Sam68 (Sellier et al. 2010). In the case of hnRNP A2/B1, evidence suggests that this protein also recruits CUGBP1, a splicing factor whose dysregulation has also been implicated in DM1 and DM2 (reviewed in Lee and Cooper 2009). Loss of Pur α causes ataxia in mice (Khalili et al. 2003) that would be consistent with sequestration of this protein contributing to FXTAS. In addition to binding to CGG-RNA, Pur α binds Rm62, an RNA helicase involved in the nuclear export of a subset of mRNAs (Qurashi et al. 2011). Expression of CGG-RNA in flies is associated with the nuclear accumulation of mRNAs involved in stress and immune responses that are normally exported from the nucleus by Rm62 (Qurashi et al. 2011).

Many splicing defects characteristic of the loss of Sam68 and deficiencies of mature miRNAs characteristic of the loss of DGCR8 are seen in FXTAS patients (Sellier et al. 2010, 2013) and overexpression of some of the abovementioned CGG-binding proteins relieves some of the CGG-mediated toxicity in different model systems (Galloway et al. 2014; He et al. 2014; Jin et al. 2007; Qurashi et al. 2011; Sellier et al. 2013; Sofola et al. 2007). This would be consistent with a role for repeat-mediated protein sequestration in FXTAS pathology.

As discussed in more detail in Chap. 6, RAN translation of *FMR1* transcripts is thought to initiate upstream of the repeat and results primarily in the generation of FMRpolyG, a novel protein containing a large polyglycine tract but lacking the FMRP sequence (Todd et al. 2013). However, some translation in the alanine frame is also seen in cell culture (Todd et al. 2013). Some RAN translation is also seen in normal cells. Since translation of small upstream ORFs leads to reduced translation of the downstream ORF, it has been suggested that this translation may be a normal aspect of the regulation of FMRP in humans. Antibodies to FMRpolyG stain most of the intranuclear neuronal inclusions seen in cells expressing this protein (Todd et al. 2013). These inclusions are also ubiquitin-positive. Similar inclusions are also seen in a variety of non-CNS tissue including kidney, heart, and adrenal organs (Buijsen et al. 2014). RAN translation in cell-based reporter systems is associated with impairment of the ubiquitin proteasome system thus implicating protein misfolding in the etiology of the CGG-repeat toxicity (Oh et al. 2015).

Similar FMRpolyG-positive inclusions are seen in reporter cell lines, in flies and in one of two PM knock-in (KI) mouse models. The FMRpolyG-positive mouse model contains a targeted insertion of the human 5′ UTR into the 5′ end of the murine *Fmr1* gene that removed the endogenous translation termination codon as illustrated in Fig. 11.2 (Willemsen et al. 2003). In the second mouse model the murine translation termination site was preserved as illustrated in Fig. 11.2 (Entezam et al. 2007) and thus these mice are FMRpolyG-negative (Todd et al. 2013). While these mice have more inclusions than wild-type mice, they have far fewer than the FMRpolyG-positive mice. Nonetheless, the FMRpolyG-negative

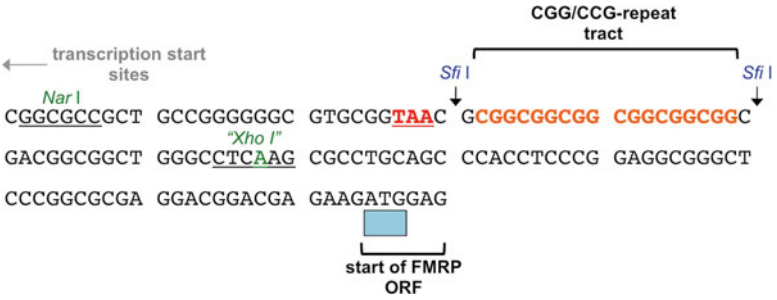

Fig. 11.2 Sequence of the 5′ end of the mouse *Fmr1* gene illustrating the difference in the two currently available KI PM mouse models. One mouse model was generated by modifying the mouse sequence "CTCAAG" to generate an *Xho* I site and then replacing the *Nar I-Xho I* fragment with the corresponding fragment from a human PM allele that lacks the TAA termination codon (Willemsen et al. 2003). This model thus produces FMRpolyG protein. The second mouse model was generated by introducing two non-homologous *Sfi* I sites so as to flank the murine repeat tract and enable the replacement of the endogenous repeat with a longer repeat (Entezam et al. 2007). These mice retain the murine stop codon and are thus FMRpolyG-negative

mice show evidence of Purkinje cell dropout and other pathological changes consistent with neurodegeneration (Entezam et al. 2007). This raises the possibility that both RNA-mediated protein sequestration and RAN translation contribute to the symptoms of FXTAS.

Since many of the manifestations of protein sequestration and RAN translation that are seen in brains of patients with FXTAS and in the CNS of various animal models are also seen in non-CNS cells in tissue culture (Oh et al. 2015) and in organs like kidney, adrenal glands, and heart (Buijsen et al. 2014), there is no reason to think that the consequences of these processes are limited to the CNS. Furthermore, a number of general cellular abnormalities that have been attributed to the PM allele could, in principle, be deleterious to a variety of cell types. This includes mitochondrial dysfunction (Hukema et al. 2014; Ross-Inta et al. 2010) perhaps arising from altered zinc transport and disrupted mitochondrial protein processing (Napoli et al. 2011), as well as abnormalities in the expression and organization of lamin A/C (Garcia-Arocena et al. 2010). It is thus possible that the expression of PM alleles affects the viability of many cell types including ovarian cells in the same or similar ways.

Lessons from Rodent Models of FXPOI

Since the mRNA produced from the PM allele is thought to be responsible for FXPOI, the expression pattern of FMRP in ovaries might provide important clues as to where the pathology in FXPOI originates. In wild-type rodents, FMRP is found in the cytoplasm of granulosa cells, as well as oocytes at all stages of maturation, but not in interstitial cells (Ferder et al. 2013; Hoffman et al. 2012; Lu et al.

2012; Takahashi et al. 2015). There have been conflicting reports as to whether FMRP is present at higher levels in more advanced follicles than in less advanced ones (Ferder et al. 2013; Takahashi et al. 2015). However it is clear that since the *Fmr1* gene is highly expressed in all follicles, both oocytes and granulosa cells may be vulnerable to the deleterious effects of the PM transcript.

The effect of the PM on the ovary has mainly been studied in two different mouse models: the FMRpolyG-negative KI mouse model (Hoffman et al. 2012) and a transgenic model carrying a YAC with a human PM allele with 90 CGG-repeats (Peier and Nelson 2002) that has the potential to make a RAN-translated product. The YAC mice showed an increased incidence of infertility/sterility and females in both mouse models showed a reduced fecundity (Lu et al. 2012; Hoffman et al., unpublished data). In the YAC mice the reduced fecundity was associated with a later age at which the first litter was produced as well as subsequent smaller sized litters. Both mouse models have smaller ovaries compared to wild-type mice (Hoffman et al. 2012; Lu et al. 2012), but the size of the primordial follicle pool in young mice was indistinguishable from that of wild-type animals. However, in older mice a significant reduction was seen in the size of all subclasses of more advanced follicles, as well as in the number of corpora lutea. These data suggest that the premutation does not affect the establishment of the primordial follicle pool, nor does it block follicular development at a certain developmental stage or increase follicular recruitment. The KI mice had granulosa cell abnormalities including a decrease in the number of granulosa cells per follicle and in an increase in follicles in which the corona was incomplete or missing. Since granulosa cell health is essential for optimal oocyte quality, granulosa cell abnormalities could well contribute to reduced fertility and ovarian dysfunction. Both mouse models showed an increase in the number of atretic follicles and increased apoptosis was shown in the YAC mice using TUNEL staining. Interestingly, in an inducible mouse model for FXTAS extensive apoptosis in the liver is seen shortly after transcription is induced with death occurring soon after (Hukema et al. 2014). The ovarian phenotype in the KI and YAC mice is very different from that seen in *Fmr1* KO mice (Ascano et al. 2012). This difference lends support to the idea that ovarian pathology in women with the PM is not due to reduced FMRP protein levels, but is due instead to the consequences of (over)expression of CGG-repeat containing RNA, as suggested for FXTAS.

At the molecular level, increased expression of Fmr1 mRNA is seen in the ovaries of both models comparable to that seen in the brains of PM mice (Entezam et al. 2007) and in the blood and brain of FXTAS patients (Tassone et al. 2000a, b, 2007). As in the brain, the levels of FMRP were lower than normal. However, the proportion of FMRP in the nucleus of oocytes is increased in the KI mice (Hoffman et al. 2012). The YAC mice showed higher FSH and lower LH hormone levels compared to wild-type mice. In addition, expression of the LH receptor (LHR) was reduced and many LH-regulated genes were downregulated at the proestrus stage in adults (Lu et al. 2012). However, an arrest in folliculogenesis beyond the antral stage and an increase in LH characteristic of LHR knockout mice were not seen in these animals (Lei et al. 2001). Of particular interest was the fact that the phosphorylation of

Akt and mTOR (mammalian target of rapamycin) was reduced in the ovaries of the YAC mice. Such a decrease would be consistent with the fact that LH activates mTOR (Palaniappan and Menon 2010). Since the inhibition of mTOR results in reduced granulosa cell proliferation and reduced follicle growth (Yaba et al. 2008), this may account for the granulosa cell abnormalities seen in the KI mice. Activating mTOR in a *Drosophila* model for FXTAS ameliorates neurodegeneration (Lin et al. 2013) raising the possibility that it also could reduce the effects of the PM on the mouse ovary.

Interestingly, increased activation of the Akt/mTOR pathway is seen FXS and in *Fmr1* KO mice. Activation of this pathway could also cause POI since genetic inactivation of a repressor of this pathway or the chemical stimulation of this pathway results in the premature activation of all primordial follicles and thus the premature depletion of the primordial follicle pool (Li et al. 2010; Reddy et al. 2009). Thus the different ovarian phenotypes of PM and knockout mice may reflect differential effects of the expression of the PM allele and the absence of FMRP on the operation of the same pathway. This raises the question of whether the unusual U-shaped relationship between repeat number and POI seen in FXPOI (Allen et al. 2007; Ennis et al. 2006; Mailick et al. 2014; Spath et al. 2011; Sullivan et al. 2005; Tejada et al. 2008) results from the effect of the RNA-mediated decrease in the activity of the mTOR pathway being offset by the activation of this pathway resulting from the reduced FMRP translation from larger PM alleles.

The basis of the dysregulation of the Akt/mTOR pathway in the PM mice is unknown and no link has been described thus far between this pathway and either RNA sequestration or RAN translation. The presence of ubiquitin-positive intranuclear inclusions in brain is a key hallmark of FXTAS. However, as in humans (Chang et al. 2011), no inclusions have as yet been found in the oocytes or granulosa cells of either the KI or the YAC mouse models (Hoffman et al. 2012; Lu et al. 2012). This is in spite of the fact that inclusions are not confined to the CNS (Brouwer et al. 2008; Buijsen et al. 2014; Hunsaker et al. 2011; Schluter et al. 2012; Willemsen et al. 2003). The absence of inclusions in the ovarian follicle raises the question of whether these inclusions are pathological or whether pathology results from events occurring outside the ovary. The early lethality seen in the inducible mouse model of FXTAS occurs in the absence of ubiquitin-positive inclusions (Hukema et al. 2014). This lends support to the idea that inclusion formation may not be required for toxicity. Whether this reflects a smaller role for RAN translation, which accounts for most inclusions, in toxicity or whether RAN translation can cause problems even in the absence of inclusion-formation remains to be seen.

Conclusions and Future Directions

Both the YAC and FMRpolyG-negative KI mouse models show ovarian abnormalities that may provide clues to the molecular pathology responsible for FXPOI. These models show that the PM allele does not affect the development of the primordial

follicle pool at least in mice. It also does not cause specific blocks in follicular development or the premature activation of follicles. Instead the data point to a problem in granulosa cell proliferation perhaps related to inhibition of the Akt/mTOR pathway. Whether dysregulation of the same pathways occurs in women with FXPOI remains to be seen.

Many key questions remain to be answered including the primary site of FXPOI pathology and whether RNA sequestration and/or RAN translation is responsible. Since the hypothalamus-pituitary-adrenal axis is affected in KI mice (Brouwer et al. 2008), it is possible that pathology arising outside the ovary somewhere in the hypothalamus-pituitary-gonadal axis causes FXPOI. However, the fact that the PM does not affect the age at menarche in women with FXPOI, along with the fact that no stage-specific arrest in follicle development is seen in either mouse model could be consistent with a problem intrinsic to the ovary. The fact that skewing of X inactivation in women does not affect the risk of FXPOI would be consistent with this view since it suggests that pathology arises in the oocyte. Since oocyte and granulosa cell health are so intimately intertwined, it is possible that the oocyte is the primary site of the pathology and that oocyte dysfunction in turn affects granulosa cell viability thus initiating a vicious cycle that results in reduced oocyte quality and survival.

The fact that even the FMRpolyG-negative mice show some ovarian pathology suggests that RNA sequestration or some other consequence of expression of the CGG-RNA itself may contribute, at least partially, to the ovarian dysfunction seen in women with FXPOI. Mouse models using an oocyte or granulosa cell-specific inducible promoter should give decisive answers as to the question of the primary site of pathology. Primate models and germ line cells derived from embryonic stem cells or induced pluripotent stem cells derived from women with the PM may also provide systems amenable to dissection of the pathology that results.

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Chapter 12
Premutation-Associated Disorders in Childhood and Adulthood

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Abstract This chapter is focused on the medical, developmental, and psychiatric aspects of premutation-associated disorders with the exception of FXTAS and FXPOI, which are covered in other chapters. A historical perspective of our understanding of premutation involvement is presented and the spectrum of premutation involvement is reviewed. Most children with the premutation do not have significant clinical involvement and most adults do not suffer from medical or psychiatric problems although when they occur it is important for the health care provider to be aware and provide treatment. Many of the neuropsychiatric and central nervous system (CNS) findings in premutation carriers are subclinical and related to the RNA toxicity that occurs with the premutation. Why some individuals with the premutation have clinical symptoms and others do not is likely related to several factors that are described in detail. Lifestyle changes to avoid toxins and promote health are also discussed.

Keywords Fibromyalgia • Migraines • Hypertension • ADHD • ASD • Depression • Anxiety

Introduction

Fragile X-associated disorders include fragile X syndrome (FXS), fragile X-associated primary ovarian insufficiency (FXPOI), fragile X-associated tremor/ataxia syndrome (FXTAS), and a variety of additional premutation-associated disorders, including developmental, emotional, psychiatric, medical, and neurological

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problems. This chapter is devoted to the additional problems associated with the premutation beyond FXTAS and FXPOI. Many of the clinical problems associated with the premutation have taken more than two decades to be recognized. Some of the health or emotional problems, such as anxiety, depression, or hypertension, are common in the general population and so may not be immediately recognized as associated with the premutation. Large studies from multiple centers have been done to establish some of these associations with the premutation (as reviewed by Wheeler et al. 2014, 2015a) although additional studies are needed. Even the name "premutation" was created because this allele size was found to lead to FXS in the subsequent generation, but it was not associated with clinical features of FXS. The premutation has historically been defined by the CGG expansion of 55–200 repeats though the lower boundary has fluctuated from 50 to 55 over the last decade. Given the smallest CGG-repeat number known to expand to a full mutation in the next generation is 56 (Fernandez-Carvajal et al. 2009), it has been generally accepted that the premutation begins at 55 repeats (Monaghan et al. 2013; Sherman et al. 2005). Thus, the range of 55–200 repeats is based only on the risk of expansion, not the risk for high-repeat-associated disorders. Eventually, the high-risk repeat range for FXPOI, FXTAS, and other potential outcomes associated with the premutation will need to be better defined. However, these ranges may or may not be the same for all disorders, so we await more knowledge about the mechanisms through which their associated outcomes occur. There is some evidence that the gray zone, considered 45–54 CGG repeats, may modify the risk for other clinical problems, including neurodevelopmental deficits (Aziz et al. 2003; Renda et al. 2014), neurological dysfunction such as FXTAS (Hall et al. 2012; Liu et al. 2013), and reproductive problems, including primary ovarian insufficiency (Bodega et al. 2006; Bretherick et al. 2005); Chap. 10 provides further discussion of the gray zone.

The historical designation of "non-penetrant transmitting males (NTM) or carriers" was hard to change because it was used so frequently in publications in the 1980s and 1990s. The recognition of early ovarian insufficiency in carriers was accepted after the report by Cronister et al. (1991) and followed by many others demonstrating that approximately 16–20% of women with the premutation have FXPOI (Sherman et al. 2014; Sullivan et al. 2005; see Chap. 10). Subsequently, clinical involvement in some children with the premutation, including attention deficit hyperactivity disorder (ADHD), learning disabilities, autism, and even intellectual disability (ID), were reported (Aziz et al. 2003; Hagerman et al. 1996; Tassone et al. 2000b), suggesting that the *FMR1* gene product (FMRP) may be reduced to a level that leads to FXS-associated features. The report by Tassone et al. (2000a) documenting the elevation in *FMR1* mRNA levels in the premutation range, the opposite of what was expected, was game changing because it provided clear evidence of a molecular phenotype that was different from that of the full mutation. This molecular finding opened the way to consider other phenotypes of premutation involvement, particularly the emerging neurological problems in carriers leading to symptoms of FXTAS, which were first reported in 2001 (Hagerman et al. 2001) and named FXTAS in 2003 (Jacquemont et al. 2003).

Refining the definition of FXTAS to now include the gray zone and even unmethylated or mosaic individuals with either the full mutation or with the premutation in enough cells to lead to elevation of *FMR1* mRNA levels (Hall et al. 2012; Liu et al. 2013; Loesch et al. 2012; Pretto et al. 2013) has been described in Chap. 1 and in other reviews (Hagerman and Hagerman 2013, 2015; Hall et al. 2014). The phenotypic features that may need to be added to the diagnostic criteria also include neuropathy and white matter disease in the splenium of the corpus callosum as reviewed by Hall et al. (2014). However, the field has not come to consensus with how to best identify the more mild aspects of premutation involvement that may build up over years before the full diagnostic criteria of FXTAS is met, even though these prodromal problems are likely to be caused by the same or similar molecular pathological processes that eventually leads to FXTAS. All of the problems described here can be called "premutation-associated disorders," but they do not meet criteria for the diagnosis of FXTAS or FXPOI, which are described in Chaps. 1 and 10 in this volume, respectively.

The animal data, particularly those from the premutation knock-in (KI) mouse, have demonstrated that premutation involvement can begin early in development (reviewed in Hunsaker 2013). Cultures of premutation neurons have shown mitochondrial problems that impact how connections are made between neurons (Kaplan et al. 2012), and deficits in neuronal migration (Cunningham et al. 2011). Subtle visual–spatial deficits can be identified early in the mouse models of the premutation (Hunsaker 2013) although reaction time has been shown to be faster in premutation carrier women than controls (Goodrich-Hunsaker et al. 2011a, c). For most carriers, these perceptual problems have not caused difficulties that manifest clinically, and most premutation carriers are very successful in life and in intellectual endeavors. However, radiological evidence from MRI studies demonstrate gradual changes in the brain over time that are significantly different from age-matched controls, including atrophy and white matter disease and functional/activation changes throughout adult life, which may eventually progress to FXTAS in some carriers (Battistella et al. 2013; Filley et al. 2015; Hashimoto et al. 2011a, b, c; Wang et al. 2012, 2013a, b; also see Chap. 4). These radiological changes seem to parallel the gradual emergence of executive function (EF) deficits and memory problems seen in come carriers over the life span (Cornish et al. 2008). Recent studies have documented deficits in the P300 amplitude over the frontal regions that correlate with EF deficits in carrier women without FXTAS (Yang et al. 2013).

Why do some individuals develop FXTAS or have early developmental or psychiatric problems and others do not? There are many answers that may relate to individual differences in one's background genes, lifestyle habits, exposures to toxins, and environmental supports or risk factors, in addition to the variation in the manifestation of *FMR1* mutation. Both the level of FMRP and the elevation of *FMR1* mRNA levels have an impact on functioning from a cognitive and psychiatric perspective (Hessl et al. 2005, 2007, 2011). FMRP level correlates inversely with CGG-repeat number so that individuals with >150 repeats may experience lowered FMRP levels, putting them at risk for more significant developmental problems (Ludwig et al. 2014; Pretto et al. 2014). The age of onset of FXTAS and the age of

death are associated inversely with CGG-repeat number (Greco et al. 2006; Tassone et al. 2007). Preliminary studies have shown that in 20 % of individuals with the premutation who have autism, intellectual disability (ID), or neurological and/or seizure problems, a second genetic hit is likely adding to their clinical presentation (Lozano et al. 2014). Case reports have documented that exposure to toxins in the environment, including industrial toxins (Paul et al. 2010), excessive alcohol (Muzar et al. 2014), or opioids (Muzar et al. 2014, 2015), may lead to progression of neurological symptoms. The premutation may increase vulnerability of the CNS and peripheral nervous system (PNS) because the premutation neuron dies more easily compared with control neurons, as shown in culture (Chen et al. 2010). Therefore a second genetic hit, the physiological impact of stress, or an environmental toxin may have a more deleterious effect in the brain of someone with a premutation compared to an individual without an *FMR1* mutation. The categories of premutation involvement in childhood and adulthood are discussed below.

Neurodevelopmental Problems

Despite the findings of progressive neurological changes in some adult carriers leading to FXTAS, research on children and adolescents with the premutation is scarce. While some conclusions regarding the impact of the premutation on children can be extrapolated from studies of adults, there is a significant need for more studies focusing on young individuals with the premutation. In a large survey of families affected by FXS, parents reported a higher rate of developmental and behavioral problems, particularly in boys with a premutation, compared with noncarrier children (Bailey et al. 2008). Several case studies have also found a higher than expected percentage of children with a premutation having some type of neurodevelopmental diagnosis (Chonchaiya et al. 2012; Farzin et al. 2006; Hagerman et al. 1996; Renda et al. 2014), and in some cases lower levels of FMRP have been associated with increased developmental delays (Aziz et al. 2003; Goodlin-Jones et al. 2004; Tassone et al. 2000b). However, these studies all describe outcomes for clinically referred children; studies comparing nonclinically referred children identified through cascade testing with controls have not found cognitive deficits although anxiety and ADHD symptoms are increased compared with controls (Cordeiro et al. 2015; Farzin et al. 2006; Jabbari et al. 2015; Myers et al. 2001).

Because the premutation is rarely diagnosed in infancy, there are currently only two known studies that have documented early developmental trajectories in the premutation. Gallego et al. (2014) reported visual processing deficits in 14 babies with the premutation relative to non-affected controls. The premutation infants were more like infants with a full mutation or those with Down syndrome in terms of their likelihood of having these deficits, despite higher overall developmental scores than the other two groups. In a recent study, 25 infants identified with a premutation through a newborn-screening pilot study (Wheeler et al. 2015b) were compared to screen-negative infants and no differences were found on measures of broad devel-

opment or functional skills. However, infants and toddlers with the premutation were significantly more likely to have elevated scores on measures of sensory sensitivity, social processing, and measures of early autistic spectrum disorder (ASD) symptomology than their non-affected peers. These two studies suggest there may be very early markers of sensory processing and executive dysfunction present as young as infancy, which may develop into higher risk for the social–emotional and mild cognitive challenges described in some older carriers (Chonchaiya et al. 2012; Cornish et al. 2008; Farzin et al. 2006; Goodlin-Jones et al. 2004; Grigsby et al. 2014; Loesch et al. 2003c; Losh et al. 2012; Sterling et al. 2013), as well as in studies of premutation mice (Berman et al. 2014; Hunsaker 2013).

Why these problems develop is likely related to subtle changes in the neurobiology of the premutation state. Some differences may be present during development, and other changes can develop in adulthood and worsen with aging. Premutation mouse studies demonstrate deficits in spatial temporal ordering by 12 weeks of age (Hunsaker et al. 2010). In patient studies using transcranial magnetic stimulation, young adult asymptomatic women with the premutation have shown GABAergic deficits of cerebellar inhibition and intra-cortical inhibition compared to age-matched controls (Conde et al. 2013). These findings are consistent with the early emergence of deficits in the cortico-cerebellar pathways that may affect EF, working memory, and attention/inhibition (Wang et al. 2013b). Kraan et al. (2014) found deficits in dual task performance involving gait analysis with a working memory task of counting backwards in young adult women with the premutation compared with controls. Although each task does not independently show deficits in young premutation carriers, the combined task will bring out more subtle deficits in attentional interference involving cortico-cerebellar pathways, which functionally connect higher cognitive and motor networks. Similar deficits were found in studies of brain volume, activation, and diffusion tensor imaging by several researchers in comparing asymptomatic carriers vs. controls (Battistella et al. 2013; Hashimoto et al. 2011a, b, c; Hippolyte et al. 2014; Kim et al. 2014; Wang et al. 2013b). Hippolyte et al. (2014) documented significant motor-planning problems and deficits in verbal encoding and retrieval in asymptomatic carrier men compared to controls. The deficits also correlated with white matter changes on diffusion tensor imaging (DTI) in the right dorsal lateral prefrontal cortex and in the hippocampal fimbria, respectively. Because the changes were present in the young adult carrier men and did not worsen with age, they favored a neurodevelopmental timing for these problems. Similar conclusions were reached in studies of asymptomatic carriers by Battistella et al. (2013). In young adult men with the premutation, Wong et al. (2015) neither found EF deficits nor attention problems; in contrast, their attentional blink studies found enhanced attention compared with controls. However, they did detect impairment in a working memory task of letter-number sequencing in premutation men compared with controls (Wong et al. 2015). In older adult, asymptomatic, carrier women, mean age of 55 years, Yang et al. (2013) detected decreased frontal P300 amplitudes that correlated with EF deficits on neuropsychological testing. Therefore, both neurodevelopmental deficits that are usually subclinical can combine with aging deficits that are significantly different than controls, leading to enhanced problems in EF in aging (Cornish et al.

2008, 2011). However, more natural history studies are needed to better understand the developmental trajectories of neurocognitive risks for premutation carriers.

Psychiatric/Cognitive Issues

Several studies have been conducted among children and adults who carry the premutation allele to assess neuropsychological and neuropsychiatric outcomes. However, results across these studies are inconsistent, likely due to variability in study design, such as recruitment and control populations. In addition, many studies could be confounded due to the inclusion or exclusion of probands, older individuals (over 50) who carry the premutation, or women who carry the premutation and have a child with FXS. Thus, any significant outcomes detected in these studies could be due to the inclusion of individuals with symptoms of FXTAS or to the psychosocial impact of caring for a child with special needs, rather than a general impairment associated with the premutation. Nevertheless, more recent studies have begun to address these confounding issues and are working to characterize the potential expanded range of clinical features associated with the premutation allele.

Typically, children who are carriers present with normal intellectual abilities though several cases of ID in children with the premutation have been reported (Aziz et al. 2003; Bailey et al. 2008; Chonchaiya et al. 2012; Clifford et al. 2007; Tassone et al. 2000b). However, increased rates of psychiatric problems, including anxiety, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD) have been reported in children with the premutation compared to controls, particularly among boys.

In a nationwide survey of over 1200 families affected by FXS, parent reports of their children found that among 256 premutation carriers (57 boys and 199 girls), 45 % of boys over the age of six had attention problems, 36 % had anxiety, 32 % had developmental delay, 30 % had ADHD, and 19 % had aggression. The girls who were carriers demonstrated fewer problems; however, 14 % had attention problems, 31 % experienced anxiety, 6 % had developmental delays, 3 % had ADHD, 4 % had aggression, and only 1 % were diagnosed with autism (Bailey et al. 2008). This study, however, did not use standardized diagnostic testing and did not separate those carriers who were the probands of the family and those who were identified by cascade testing in a family once a proband was diagnosed. Other studies have identified a much higher rate of involvement of ASD, ADHD, ID, anxiety, and seizures in those carriers who present as the proband compared to carriers identified through cascade testing (Chonchaiya et al. 2012; Cordeiro et al. 2015; Farzin et al. 2006). Seizures have been reported in 8–16 % of carriers (Bailey et al. 2008; Chonchaiya et al. 2012), and Chonchaiya et al. (2012) found that the presence of seizures correlated with ASD and ID, suggesting that an additional factor may be leading to both disorders. In support of this "second hit" hypothesis, Lozano et al. (2014) found that 20 % of premutation carriers with ID, ASD, or neurological problems had a second genetic problem identified by microarray testing or whole

genome sequencing. Therefore, such testing is indicated when severe problems such as ID, ASD, and seizures are seen in premutation carriers.

In a recent study by Cordeiro et al. (2015), 35 children and young adults (27 males), ages 5–23 years (mean age of 11 years; 20 probands and 14 non-probands), and 31 controls were evaluated with the Anxiety Disorders Interview Schedule for DSM-IV (ADIS). They found that 70 % of carriers met criteria for at least one anxiety disorder, most frequently generalized anxiety disorder (37.1 %), specific phobia (31.4 %), social phobia (28.6 %), or obsessive compulsive disorder (22.9 %) vs. 22.6 % of controls and compared with 9.8 % of the general population. Anxiety disorders were significantly higher if the patient presented as the proband for the family (94.7 %), had ID (81.8 %), or was a male (70.6 %). However, even the non-proband premutation carriers had a significantly higher rate of an anxiety disorder (40 %) than the general population (9.8 %). Similar results were found in a study of 35 non-proband girls with the premutation (mean age 8 +/− 5) and 33 control girls (mean age 9 +/− 6) who showed a higher rate of anxiety by parental history (premutation 62.9 % and control 16.7 %; $p = 0.001$), shyness (premutation 54.3 % and 23.3 % control; $p = 0.013$), and hyperactivity (premutation 22.0 % and control 3.3 %; $p = 0.031$), compared with controls (Jabbari et al. 2015).

Among studies in adult populations, evidence indicates that the premutation allele is not associated with significant impairments in general intelligence compared with control populations (Allingham-Hawkins et al. 1996; Bennetto et al. 2001; Reiss et al. 1993; Riddle et al. 1998; Thompson et al. 1994) although these studies have not included those who present with clinical problems. However, studies assessing specific cognitive domains, such as EF and memory, have reported varied conclusions. While some studies have reported no specific cognitive deficits associated with the premutation (Bennetto et al. 2001; Hunter et al. 2008, 2010; Thompson et al. 1994), other study populations of individuals with the premutation have performed significantly worse on specific aspects of cognitive functioning (Brega et al. 2008; Cornish et al. 2008; Franke et al. 1999; Grigsby et al. 2008, 2014; Kraan et al. 2014; Loesch et al. 2003a, c; Moore et al. 2004a, b) though only a subset of carriers appear to be impacted and the deficits detected are typically mild.

Though executive functioning, a complex cognitive domain that involves planning and organization, is typically impaired in men with FXTAS (Brega et al. 2008), a series of studies have reported EF deficits among men with the premutation who do not meet the diagnostic criteria for FXTAS (Brega et al. 2008; Cornish et al. 2008, 2009; Grigsby et al. 2008, 2014; Hunter et al. 2012a, c; Kogan et al. 2008). Specifically, impairments in inhibitory control (Cornish et al. 2008, 2011) and working memory (Cornish et al. 2008, 2009, 2011; Grigsby et al. 2008) have been characterized. Among these studies, these deficits appear to begin early in adulthood and display an age-related decline, with men with greater than 100 CGG repeats being at the highest risk (Cornish et al. 2008, 2011; Hunter et al. 2008). Similar deficits in EF have been recently reported among study populations of women who carry a premutation allele, including deficits in inhibition (Kraan et al. 2014) and working memory (Shelton et al. 2015).

Additional cognitive domains have also been reported to be impaired among premutation carriers. Specifically, impairment in declarative learning and memory have been seen among men who carry the premutation (Grigsby et al. 2008; Grigsby et al. 2014; see Chap. 3), while deficits in visuospatial processing (Goodrich-Hunsaker et al. 2011a; Hocking et al. 2012), verbal abilities (Allen et al. 2005; Franke et al. 1999; Losh et al. 2012), and arithmetic (Franke et al. 1999; Lachiewicz et al. 2006) have been reported among women who carry the premutation. Deficits in attention have also been noted (Cornish et al. 2008; Hunter et al. 2012a, c; Kogan et al. 2008; Kraan et al. 2013). Cornish and colleagues reported lower scores among men with a premutation for selective attention but not sustained attention, indicating that their ability to maintain attention on a task was only impaired in the presence of distracting stimuli. In addition, self-reported symptoms associated with ADHD have been reported to be elevated in women who carry the premutation though these symptoms appear to be limited to inattention, memory, impulsivity, and emotional lability (Hunter et al. 2012a, c; Kraan et al. 2013). Furthermore, visual–spatial deficits and enumeration deficits have been reported in women with the premutation (Goodrich-Hunsaker et al. 2011a, b; Wong et al. 2015).

Psychiatric and mood disorders have been extensively studied in carriers of premutation alleles, and findings suggest that women tend to be more vulnerable to these outcomes. While the results of some studies did not indicate any evidence of an elevated risk for psychiatric or mood disorders (Reiss et al. 1993), others indicate that premutation carriers are vulnerable to these disorders (Bailey et al. 2008; Besterman et al. 2014; Bourgeois et al. 2011; Franke et al. 1998; Hessl et al. 2005; Hunter et al. 2012a; Johnston et al. 2001; Roberts et al. 2009; Seritan et al. 2013; Sobesky et al. 1996). However, the types and severity of disorders varies across these studies.

Higher rates of anxiety and depression have been reported among women who carry a premutation allele than would be expected in the general population (Bourgeois et al. 2011; Franke et al. 1998; Johnston et al. 2001; Roberts et al. 2009; Thompson et al. 1994). However, some studies reported similar rates among women with a premutation who were mothers of children with FXS and women who were mothers of children with special needs (e.g., ASD) (Franke et al. 1996; Rodriguez-Revenga et al. 2008). Impairments in social interactions have also been reported, including social cognition (Cornish et al. 2005), interpersonal sensitivity (Johnston et al. 2001), and elevated distress experienced in social situations (Bourgeois et al. 2011; Franke et al. 1998; Kraan et al. 2013).

Recent studies indicate that these elevated symptoms associated with anxiety, depression, and social anxiety could, in part, be related to the psychosocial stress of raising a child with FXS (Hartley et al. 2012; Hunter et al. 2012b; Kenna et al. 2013; Seltzer et al. 2012b). Hartley and colleagues reported that women with a lower activation ratio (defined as the percentage of active X chromosomes with the normal *FMR1* allele) show an atypical cortisol stress response to negative child behavior, indicating an impact of stress on neuroendocrine functioning. Further, Seltzer and colleagues (2012b) reported higher rates of anxiety and depression in mothers of a child with FXS who had experienced a stressful life event the year before, with women carrying

premutation alleles in the mid-range being the most at risk. Hunter and colleagues (2012a) showed an association with endogenous stress response to predict the level of distress in social situations, which was specific to mothers of children with FXS. Thus, many of the emotional and neuropsychiatric outcomes among women who carry the premutation allele can be exacerbated by the psychosocial impact of raising a child with FXS. However, the increased incidence of psychopathology begins before a woman with the premutation has children (Roberts et al. 2009; Seritan et al. 2013). Those mothers who experience aggression from their child, usually in the form of hitting, are at the highest risk for stress and depression (Bailey et al. 2012).

Given the variability in results across studies, the nature and extent of these neuropsychological and psychiatric deficits are still not fully understood and warrant further investigation. However, it is clear that not all individuals with the premutation will manifest these problems. Recent studies have begun to identify factors that might lead to increased vulnerability to these outcomes, including the contribution of genetic and genomic variation outside of *FMR1* (Hunter et al. 2012a; Lozano et al. 2014), hormonal dysfunction among women (Hunter et al. 2010), and specific premutation repeat ranges (Loesch et al. 2015; Seltzer et al. 2012a). In addition, premutation carrier men have been reported to have higher rates of alcohol abuse compared with control men without the premutation (Kogan et al. 2008). A similar finding of higher alcohol abuse in carrier men was seen by Dorn et al. (1994) in interviews with daughters of carrier men compared to control men. Perhaps alcohol use is a form of self-medication in these men who experience stress, psychiatric problems, and ADHD symptoms. However, such use may worsen white matter disease and perhaps lead to earlier onset of FXTAS (Muzar et al. 2014). Longitudinal studies to track phenotypic patterns over time would help elucidate risk factors for the cognitive or emotional impairments reported among some premutation carriers. These risk factors could include the psychosocial stress of caring for a child with FXS or a parent with FXTAS (Iosif et al. 2013), early signs of FXTAS, ovarian dysfunction, or additional genetic variation beyond *FMR1*. Further evidence is warranted to support the prevalence and impact of neuropsychological and neuropsychiatric issues among carriers of premutation alleles.

Medical and Neurological Findings Short of FXTAS

A variety of neurological problems are associated with the premutation that are not part of the FXTAS phenotype although the same process of RNA toxicity may lead to these symptoms. One example is migraines, which are significantly increased in premutation carriers compared with controls (Au et al. 2013). The presence and absence of migraines was assessed in 315 *FMR1* premutation carriers (203 women; 112 men) and 154 controls (83 women; 71 men). The authors found the prevalence of migraine in premutation carrier women (54.2 %; 110/203) was significantly higher than the prevalence of migraine in control women, which was 25.3 % (21/83, $p = 0.0001$). Likewise, the prevalence of migraine in premutation carrier men

(26.79 %, 30/112) was significantly higher than the prevalence in control men, which was 15.49 % (11/71; $p = 0.0406$). The finding occurred in premutation carrier men and women both with and without FXTAS. The presence of migraine further increases the risk of developing comorbidities, such as cognitive deficits, depression, and anxiety (Tietjen et al. 2007). There is evidence that mitochondrial dysfunction not only occurs in those with FXTAS but also to a lesser extent in those carriers without FXTAS (Napoli et al. 2012; Ross-Inta et al. 2010); migraines are also associated with mitochondrial dysfunction, so the underlying mitochondrial dysfunction of some carriers may also increase the risk for migraines (Au et al. 2013). Early identification of and treatments for migraine are available and may reduce this risk, so screening for migraine should be part of the routine evaluation of premutation carriers.

Sleep apnea is common in many neurodegenerative disorders including Parkinson disease and Alzheimer disease. The odds of sleep apnea in premutation carriers with FXTAS were found to be higher (31.4 %; 37/118) compared with both premutation carriers without FXTAS (8.6 %; 15/174) and controls (13.8 %; 17/123) in a study by Hamlin et al. (2011). The adjusted odds of sleep apnea for premutation carriers with FXTAS was 3.4 times that compared with controls (odds ratio, OR = 3.4, $p = 0.001$). Although carriers without FXTAS did not show an increased risk of sleep apnea compared with controls, these results have important implications for all carriers. Sleep apnea causes frequent periods of hypoxia, which are particularly detrimental to neuronal function (Chiang 2006) and may hasten the onset or progression of FXTAS. Premutation neurons show increased fragility and sensitivity to cell death in culture (Chen et al. 2010) and decreased oxygen uptake (Ross-Inta et al. 2010). Therefore, oxygen deprivation from sleep apnea-driven hypoxic episodes would enhance oxidative stress and RNA toxicity in neurons and increase the ATP deficit in the brain due to even less oxygen available to neuronal mitochondria. Such damage to the already fragile premutation neurons may decrease cognitive function and accelerate the progression of FXTAS (Hamlin et al. 2011). Thus, evaluation and proper treatment of sleep apnea in both premutation carriers with or without FXTAS is important for preventing additional neuronal damage and improving quality of life.

Coffey et al. (2008) showed hypertension to be significantly higher in premutation carrier women with FXTAS compared with controls, and Hamlin et al. (2012) reported the same finding in men over 40 years old. Neither study showed an increased risk of hypertension in premutation carrier men and women without FXTAS compared with controls. However, depression, anxiety, and other psychiatric problems are common in premutation carriers both with and without FXTAS (Bourgeois et al. 2009, 2011; Hessl et al. 2005) and may be related to increased blood pressure. Untreated hypertension can lead to cardiovascular complications, dementia, and stroke (Chobanian et al. 2003), and may worsen the progression of white matter disease (Gottesman et al. 2010; Sierra and Coca 2006). Hypertension and psychiatric problems that may increase blood pressure should be screened for and treated in premutation carriers without FXTAS to reduce the risk of developing FXTAS and in premutation carriers with FXTAS to help slow its progression.

Neuropathy is a common finding in premutation carriers, and Coffey et al. (2008) found neuropathy symptoms in 45 % of 128 premutation women without FXTAS, significantly increased compared with controls (11.9 %). Berry-Kravis et al. (2007) found an increase in neuropathy findings on the neurological examination of carriers vs. controls. Soontarapornchai et al. (2008) carried out nerve conduction studies in carrier men with and without FXTAS compared with controls. They found that approximately 30 % of carriers without FXTAS demonstrated abnormalities on nerve conduction studies, including temporal dispersion, prolonged F wave latencies, and sensory neuronal action potentials, demonstrating that a subset of carriers had electrophysiological neuropathic findings. Hagerman et al. (2007) reported several cases where neuropathy was the presenting feature of neurological problems that subsequently developed into FXTAS.

Medical Problems Including Immune-Mediated Disorders

The study by Coffey et al. (2008) of 146 carrier women (18 with FXTAS) compared with age-matched controls brought attention to the increased prevalence of fibromyalgia (43.8 %), hypertension (61.1 %), and thyroid disease (50 %) in premutation carrier women with FXTAS. The carriers without FXTAS demonstrated a higher rate of neuropathic symptoms (45.2 %) and muscle pain (25.6 %) compared with controls. A subsequent study surveyed 344 carrier women, ages 19–81 years, for immune-mediated disorders (IMDs) and found an overall rate of 44.7 % with at least one IMD. Of the 55 women with FXTAS, 72.7 % had at least one IMD compared with 27.8 % of controls and 46.5 % of those without FXTAS, leading to a significant increase of IMDs in women with the premutation (Winarni et al. 2012). The most common IMDs were autoimmune thyroid disease seen in 24.4 %, fibromyalgia in 10.2 %, irritable bowel syndrome in 9.9 %, Raynaud's phenomenon in 7.6 %, rheumatoid arthritis in 3.8 %, systemic lupus erythematous in 2 %, and multiple sclerosis in 1.74 %. Previous case reports of fibromyalgia (Leehey et al. 2011b) and multiple sclerosis (Greco et al. 2008; Zhang et al. 2009) have been documented in premutation carriers, and autopsy in one carrier who died of multiple sclerosis also demonstrated the pathology of FXTAS with inclusions in the neurons and astrocytes throughout the brain (Greco et al. 2008). The RNA toxicity associated with elevated *FMR1* mRNA in carriers includes a sequestration of DROSHA and DGCR8, two proteins that are necessary for the maturation of microRNAs (miRNAs), which in turn regulate the expression of genes (Sellier et al. 2013). Dysregulation of the miRNA maturation has led to lowered levels of multiple miRNAs and cytokine/chemokine imbalances that may predispose carrier women to immune-mediated problems (Careaga et al. 2014; Sellier et al. 2014). Treatment of the IMDs requires further study. If the IMD leads to hypothyroidism, the replacement of thyroid hormone is easily done if the test to detect a potential deficiency is ordered.

Some individuals with the premutation have lowered levels of FMRP, particularly those with a CGG-repeat above 120 (Ludwig et al. 2014; Pretto et al. 2014),

and they often experience connective tissue problems similar to those with the full mutation. The phenotypic features of loose connective tissue include joint laxity, which can lead to dislocation, hyperextensible finger joints, and prominent ears; these features are more common in younger carriers than controls (Loesch et al. 2003b; Riddle et al. 1998). Gastroesophageal reflux is common at all ages and can lead to esophagitis and pain. Additional GI symptoms include irritable bowel syndrome and, as the PNS neurons in the myenteric plexus and bowel wall become involved with inclusions, constipation, and swallowing problems become more problematic (Hunsaker et al. 2011; Leehey et al. 2011a). Inclusions can also occur in the heart and in the pericardial ganglia, sometimes leading to arrhythmias (including bradycardia), and sometimes requiring a pacemaker or ablation (Hagerman and Hagerman 2013; Hagerman et al. 2001; Hunsaker et al. 2011).

Sleep disturbances besides sleep apnea are common in carriers and were the most common complaints of the daughters of men who were diagnosed with FXTAS (Chonchaiya et al. 2010). Significant sleep problems were also documented on sleep questionnaires seen in a cohort of 127 carriers (mean age 67+/− 10; 39 % women), including insomnia and lower quality of sleep compared with age- and sex-matched controls (Summers et al. 2014). A significant increase in restless legs syndrome (RLS) was seen in these carriers (33.1 %) compared with controls (16.3 %), and the presence or absence of FXTAS did not change the prevalence in carriers. RLS is more common in those with anemia and iron dysregulation in the CNS, which can occur in carriers (Ariza et al. 2015). There is also evidence of enhanced iron deposition at a cellular level (Ariza et al. 2015) and on magnetic resonance imaging (MRI) (Wang et al. 2013a) in those with FXTAS. The GABA deficits reported in carrier women (Conde et al. 2013), in addition to the psychiatric problems of anxiety and/or depression, may interfere with sleep. Most carriers talk about perseverative worries or a racing mind that keeps them awake at night. Wakefulness in their children may also lead to sleep deprivation though the sleep problems continue well after the children are grown (Summers et al. 2014).

Significant pain symptoms are commonly seen in carriers, including neuropathy, arthritis, migraines, fibromyalgia, and back pain, perhaps exacerbated by connective tissue problems causing more ligamentous instability (Hagerman and Hagerman 2013; Jalnapurkar et al. 2015; Leehey et al. 2011b; Soontarapornchai et al. 2008). Cases have been reported with a central pain syndrome, enhanced sensitivity to pain from a central mechanism, in premutation carriers, particularly in women with fibromyalgia (Jalnapurkar et al. 2015; Leehey et al. 2011b). In our experience, many carriers have been started on opioids at pain clinics, and subsequent addiction is common and likely to exacerbate the progression of FXTAS because of white matter structural changes caused by the opiates (Bora et al. 2012; Muzar et al. 2014). The following is a case example:

> Case 1 presented initially at age 59 when her grandson was diagnosed with FXS. She had a CGG repeat of 76 and symptoms of a neuropathy in both lower extremities, including numbness, tingling, and pain at night. She also had a history of migraine headaches beginning in her mid-20s and occurring weekly until age 40 when she went through menopause. She was hypertensive since age 45, and her hypertension was well controlled by lisinopril. She had a

benign thyroid tumor removed in her 20s and had been on levothyroxine since. Although gabapentin was recommended for her neuropathy pain, she went to a pain clinic and was placed on oxycodone 60 mg long-acting three times a day, and had remained on this dose for the last 12 years. Her bilateral knee pain led to several arthroscopies and eventually knee replacement bilaterally under general anesthesia.

She was seen again at age 68 to assess her premutation status, and she had been using a walker daily over the last 5 years after her knee replacement surgery. Since age 66, she had intermittently used a wheelchair. She slept for several hours each day and was depressed, weak with poor muscle strength, incontinent of urine, and experiencing cognitive decline; she frequently dropped things, had falls, and had unsteady handwriting. She had severe osteoporosis. She smoked one pack of cigarettes per day since young adulthood and refused to stop. On examination, she was severely ataxic, weak in all four extremities, had absent vibration sense and pinprick sensation in the lower extremities, and was hyporeflexic in all extremities. She had severe scoliosis and kyphosis, and experienced pain to the touch in the back and neck. She had only a subclinical tremor and was unsteady with pencil and paper tasks. Her MRI demonstrated severe global atrophy, a thin corpus callosum, and white matter disease in the splenium, pons and periventricular area.

She likely has FXTAS, and her rather rapid downhill decline at a relatively early age in her 60s is likely related to her addiction to opioids and cigarettes, depression, lack of exercise, and perhaps multiple general anesthesia exposures. Her primary health care provider was unaware of the premutation association with hypertension, depression, neuropathy, migraines, and FXTAS. These patients with multiple problems related to the premutation need providers who are aware of the big picture of premutation involvement, to encourage lifestyle changes to avoid toxins, stress, and depression, and to carry out preventative interventions to avoid or stall the onset of FXTAS (Polussa et al. 2014).

Summary

This chapter has summarized the medical, developmental, and psychiatric problems that individuals with the premutation sometimes experience during their lifetime, with the exception of FXTAS and FXPOI that are covered in Chaps. 1 and 10, respectively. The majority of these problems respond well to medical/psychiatric treatments. However, the recognition of these problems may often be delayed unless the physician knows that these conditions can be associated with the premutation. Regular screening through the medical history or with diagnostic testing, such as screening for depression or anxiety, blood pressure monitoring, or periodic thyroid function studies, will detect problems that can respond to treatment (Polussa et al. 2014). There are benefits to early treatment; for instance, timely treatment of hypothyroidism, sleep apnea, or hypertension will avoid the medical and neurological complications that can arise from an untreated disorder (Coffey et al. 2008; Hamlin et al. 2011, 2012). Knowing that one has the premutation may lead to avoidance of negative lifestyle influences, such as addiction to drugs or alcohol, which may further exacerbate a cycle of oxidative stress, cellular damage, and apoptosis, or neuronal cell loss (Muzar et al. 2014, 2015; Polussa et al. 2014). Promoting healthy lifestyles, including daily exercise, taking antioxidants, eating healthy foods, avoiding toxins, using stress reduction techniques, and treating anxiety or depression, may lead to far fewer of the aging problems associated with the premutation (Polussa

et al. 2014). These interventions may possibly delay the onset of FXTAS though this has yet to be proven. The opposite appears to be true however; an unhealthy lifestyle with excessive use of alcohol, opioids, or other toxins has been shown to lead to earlier onset of FXTAS (Martinez-Cerdeno et al. 2015; Muzar et al. 2014, 2015; Paul et al. 2010). In studying the development of FXTAS over time in premutation carriers, a variety of stressors, medical and psychiatric problems may build up and appear to precipitate or influence the onset of FXTAS (Jalnapurkar et al. 2015). Prophylactic interventions in lifestyle changes and early treatments make the buildup of medical/psychiatric problems less likely to lead to FXTAS. New treatments for anxiety, stress, and inhibitory deficits are being developed, and controlled trials are in order to further our understanding of the benefits of early treatment. The use of biomarkers, including psychophysiological studies such as the inhibitory deficits in eye-tracking studies (Wong et al. 2015), will help to guide the benefits of early treatment for premutation carriers.

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Chapter 13
Genetic Counseling for FXTAS and Fragile X-Associated Disorders

Louise W. Gane and Liane Abrams

Don't let a sense of desperation overtake you. You can have a good and happy life despite FXTAS. Care for your loved one, but don't forget to take care of yourself as well. … look for and cultivate something good in each day. Keep connected to your family and friends and build a support system. Remember to keep your sense of humor.

—Marilyn Darwin, wife of Richard (deceased)

Abstract Since its identification in 2001, awareness of fragile X-associated tremor/ataxia syndrome (FXTAS) and other fragile X-associated disorders (FXD) has increased. The number of patients and families impacted by these conditions continues to grow. Families with and without a known history of fragile X syndrome (FXS) are impacted, as the FXTAS diagnosis may be the first FXD diagnosed in the family. Patients are usually identified based on their neurological findings such as intentional tremor, balance and gait difficulties, cognitive impairment, and brain MRI abnormalities.

Keywords Genetic counseling • Fragile X • FXTAS • Psychosocial

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Introduction

Since its identification in 2001, awareness of fragile X-associated tremor/ataxia syndrome (FXTAS) has increased. The number of patients and families impacted by the condition continues to grow. Families with and without a history of fragile X syndrome (FXS) are impacted, as the FXTAS diagnosis may be the first fragile X-associated disorder (FXD) diagnosed in the family. Patients are usually identified based on their neurological findings such as intentional tremor, balance and gait difficulties, cognitive impairment, and brain MRI abnormalities. Genetic counseling is important to address issues related to FXTAS in males and females as well as *FMR1* mutations in general.

The diagnosis of FXTAS has many ramifications. Psychological and emotional issues are experienced by both the patient and the family. The patient also has to cope with the understanding that the gene mutation responsible for FXTAS is inherited and that other family members may also be at risk for FXTAS and *FMR1*-associated disorders (Mcconkie-Rosell et al. 2007). Therefore, the diagnosis of FXTAS cannot be made in isolation, and genetic counseling is crucial in addressing the needs of the patient, spouse, children, and other family members. The role of the genetic counselor is to address the molecular and hereditary aspects of FXTAS, help the family deal with the diagnosis, and attend to feelings of anger, grief, depression, isolation, and guilt. Also, it is the genetic counselor who will often identify family members at risk to carry the *FMR1* gene mutation and coordinate cascade testing of these relatives (Bourgeois et al. 2009).

Since the identification of the fragile X (*FMR1*) gene in 1991, the molecular characterization has led to better understanding of how the gene, the messenger ribonucleic acid (mRNA), and the fragile X mental retardation protein (FMRP) function. FXTAS is understood to be the result of a toxic gain of function attributed to increased levels of mRNA produced in the cells of those who carry the *FMR1* premutation (55–200 CGG repeats) (Hagerman and Hagerman 2004; Tassone et al. 2000). However, many questions regarding the gene, mRNA, and FMRP remain unanswered by research, and these unanswered questions can provide challenges to the genetic counselor. Some unanswered questions related to FXTAS include expressivity and penetrance in males and females (see Chap. 2) as well as the progression of the disease (Jacquemont et al. 2003; Leehey et al. 2007; Tassone et al. 2007).

The genetic counselor needs to be informed and to keep abreast of new and changing information when meeting with the patient with FXTAS. It is important that the genetic counselor is able to present information related to FXTAS in a comprehensive and sensitive manner, recognizing that the patient and his/her spouse may be overwhelmed with the complexity and amount of information shared with them. Therefore, it is critical that appropriate time be given to the family during the genetic counseling session to help them to understand all aspects and ramifications of the diagnosis of FXTAS. This may involve more than one visit with the genetic counselor.

Families who are being seen by a genetic counselor have counseling needs beyond the molecular, hereditable, and medical information. These needs may include medical management, prognosis, long-term care, end-of-life issues, and emotional support. It is the genetic counselor to whom many families will often turn for guidance. Therefore, it is important for the counselor to be comfortable in dealing with these sensitive issues and be able to provide the appropriate recommendations and referrals. In addition, the spouse may seek validation of, and support for, concerns that have been previously unaddressed or unspoken, as well as the emotions and personal psychological stressors that result from having a spouse with FXTAS. Caregiver burden is well documented in Alzheimer disease and documentation of the similar burden associated with FXTAS is reported (Cameron et al. 2002, 2006; Grunfeld et al. 2004; Seritan et al. 2008). It may also be the genetic counselor who will assume the role of providing long-term support for the patient and spouse. However, the genetic counselor cannot function in isolation and therefore should be able to identify and reach out to other professionals such as psychiatrists, psychologists, social workers, and other team members to address the needs associated with the diagnosis of FXTAS (Gane et al. 2005).

Molecular Aspects

FXTAS occurs in individuals with an *FMR1* premutation. The *FMR1* gene is located on the X chromosome at locus Xq27.1. The *FMR1* gene is formally named the "fragile X mental retardation" gene because, in the full mutation form, it is responsible for FXS, the most common form of *inherited* intellectual disability (Penagarikano et al. 2007) and single gene cause of autism (Belmonte and Bourgeron 2006. The FMR1 gene has a trinucleotide CGG repeat region in the 5′ untranslated region. In the typical or normal FMR1 gene, there are approximately 5–44 CGG repeats. The "gray zone" or intermediate alleles are considered to be CGG repeats numbering between 45 and 54. In those with an FMR1 "premutation," there are 55–200 CGG repeats and these individuals are referred to as "carriers," meaning they "carry" an unstable form of the FMR1 gene that has the potential to expand in future generations to a full mutation, with over 200 CGG repeats. Repeat mosaicism refers to when both the *FMR1* premutation and full mutation are present in the same individual. Methylation mosaicism refers to full mutation alleles being unmethylated in some cells and in others methylated in the same individual. For more comprehensive information regarding the molecular background of the FMR1 gene mutation and the molecular causation of FXTAS, see Chap. 6.

FXTAS is caused by a premutation in the FMR1 gene, which includes those individuals with an allele size of 55–200 CGG repeats. It is estimated that at least 33 % of males and 8–16 % of females with an FMR1 premutation will develop symptoms of FXTAS (Coffey et al. 2008; Hall et al. 2014; Jacquemont et al. 2004a). Other phenotypes associated with the FMR1 premutation include fragile

X-associated primary ovarian insufficiency (FXPOI) in females as well as anxiety, depression, obsessive compulsive disorder, and an increased risk for thyroid dysfunction (Hessl et al. 2006; Loesch et al. 2015; Roberts et al. 2008). Recent studies also reveal increased rates of immune-mediated diseases including thyroid disease, fibromyalgia, hypertension, migraines, neuropathy, and vestibular issues (Wheeler et al. 2014). A small subset of children with the premutation, particularly males, may present with developmental disorders including autism spectrum disorders, attention-deficit hyperactivity disorder (ADHD), learning disabilities (Aziz et al. 2003; Farzin et al. 2006), and features of FXS in those with large (over 150 CGG repeats) premutation alleles (Goodlin-Jones et al. 2004; Hagerman 2006). Fragile X-associated primary ovarian insufficiency (FXPOI) is a spectrum of ovarian dysfunction, including infertility, irregular menses, elevated follicular stimulating hormone (FSH) levels, decreased anti-Mullerian hormone, and premature ovarian failure (cessation of menses before 40), and occurs in approximately 22 % of females with an FMR1 premutation (Sherman et al. 2007).

Intermediate alleles of 45–54 CGG repeats occur in approximately 2 % of individuals in the general population (Curlis et al. 2005). Though stable in many families, instability or expansion by one or more CGG repeats due to the presence or absence of AGG interruptions can occur and is likely be the mechanism for gradual expansion to a premutation and eventually to a full mutation over a number of generations (Nolin et al. 2003, 2013; Yrigollen et al. 2014). It is currently unknown if there is any definitive clinical finding or disease risk associated with gray zone alleles, and this is a topic of active investigation.

Rarely, FXS has been reported in individuals with deletions or point mutations in the FMR1 gene (Grasso et al. 1999). Deletions and point mutations are considered to be sporadic and have a different molecular etiology than the full mutation or expansion of the FMR1 allele (Pozdnyakova and Regan 2005).

Inheritance

FXTAS and other *FMR1*-associated disorders follow the laws of X-linked inheritance and include both clinical and molecular aspects. Any male who is born to a possible or confirmed *FMR1* premutation carrier mother and presents without features of FXS has the potential to be a premutation carrier and at risk for FXTAS as well as for passing on the premutation to all of his daughters (Nolin et al. 1996; Penagarikano et al. 2007).

Given the FMR1 gene is on the X chromosome, all males with FXTAS will have received the FMR1 gene from their mothers. Therefore, there may be other maternal relatives of the male with FXTAS who are at risk for FXTAS as well as FXPOI and offspring with FXS. This would include the male patient's brothers, sisters, maternal grandparents, aunts, uncles, cousins, and their offspring. All daughters of the male patient with FXTAS will be FMR1 premutation carriers and thus at risk for FXPOI and to a lesser degree, FXTAS, and to have offspring with FXS.

The risk for a female carrier to have a child with FXS is dependent on the number of CGG repeats and the number of AGG interruptions within the CGG tract (see last paragraph) and the gender of the child. Lastly, recent research studying the possible maternal age affect on allele stability has proposed an increased risk of instability with each maternal year (Nolin et al. 2015; Yrigollen et al. 2014). The risk for a given premutation to expand to a full mutation in the offspring is given in Nolin et al. (2003). If a female inherits the full mutation, she will have a 50–70 % risk of having a slow or borderline I.Q. and manifesting features of FXS and a 30–50 % to have a normal intellect but display emotional and/or behavioral difficulties (Keysor and Mazzocco 2002). Virtually, all males with a full mutation and fully methylated will be affected with FXS though there is a range of physical, behavioral, and cognitive features (Clifford et al. 2007; Cornish et al. 2004). Males with the full mutation and low levels of methylation may function within society, hold a job, be married and have children; however, display irritability, psychiatric features, erratic behavior and are not identified until their male grandchild is diagnosed with FXS (Basuta et al. 2015).

Prenatal diagnosis for a couple when the male is an FMR1 premutation carrier is available and is becoming increasingly requested. Concern in male premutation carriers is being seen due to the increasing awareness of female premutation associated findings.

Females with FXTAS may have received the premutation from either parent as either the maternal or paternal X may carry the premutation allele. It is recommended that, if available, one or both of the parents of any newly diagnosed female carrier be tested to identify the source of the premutation. This will allow for genetic counseling and cascade testing for at-risk relatives. All children of a female with FXTAS (or an FMR1 premutation) are at 50 % risk to inherit an FMR1 pre- or full mutation. Since all daughters of the male patient with an FMR1 premutation will be obligate carriers, their children are at 50 % risk to inherit an FMR1 pre- or full mutation.

Diagnostic Testing

The molecular diagnosis of FMR1-associated disorders is a complex topic. Several techniques are available to determine repeat size status.

The FMR1 DNA test is used to test for fragile X syndrome and FXTAS and other FMR1-associated disorders. This test evaluates the number of CGG repeats within the FMR1 gene on the X chromosome. For a thorough discussion of the molecular genetics of FMR1-associated disorders, see Chap. 6.

Commonly, individuals with an FMR1 premutation are ascertained as a result of a relative with fragile X syndrome or due to symptoms of an FMR1-associated disorder such as FXTAS or FXPOI. Testing is then performed to determine which side of the family is at risk to be FMR1 carriers and thus at risk for an FXD. Genetic testing may also confirm a diagnosis in the adult relatives with symptoms of FXTAS and/or FXPOI. Test results should always be interpreted in the context of the family and clinical history of the patient.

Testing is performed on genomic DNA isolated from peripheral blood leukocytes, but prenatal DNA testing is also available at select laboratories using amniocytes or chorionic villus cells. It is important to verify with the laboratory what type of sample is needed and the details of how the test is performed. *FMR1* testing generally costs approximately $300–400 and does not include a general karyotype, professional and procedural fees, or shipping costs. Results are usually available in to 2–3 weeks. The gold standard molecular methodology for the diagnosis of Fragile X syndrome is represented by a combination of PCR and Southern blot analysis (Tassone 2015). Polymerase chain reaction (PCR) is used to determine, within several CGG repeats, the number repeats present within each FMR1 allele particularly in the normal, intermediate, and premutation range. There are a number of PCR techniques available that vary in the use of probes but all are specific to the CGG repeat region of the gene. PCR can be performed quickly and provides an estimate of alleles that are of normal, intermediate, or premutation size. As CGG repeat size obtained from most forms of PCR is only an approximation to within several CGG repeats, repeat size may vary slightly depending on the laboratory techniques. Most PCR techniques cannot determine repeat size for large repeat expansions, such as those that occur with the full mutation, because the sample may fail to amplify at this size. This may be particularly problematic in females who appear homozygous for an identical repeat size, because there is the possibility that the second allele may be a large premutation or full mutation that failed to amplify, even in the absence of clinical signs and symptoms that correlate with these repeat sizes. In addition, most PCR techniques do not provide any information about methylation status. In these situations, Southern blot analysis is necessary.

Rapid PCR technique have now been developed and available and recommended for use in large populations (Tassone 2015). This technique can screen both males and females for premutation and full mutation expansions using smaller amounts of DNA, such as that found in one blood spot. These techniques are currently in use in diagnostic laboratories and may become routine for large-scale population screening, such as in newborn screening or for those at high risk of an FMR1-associated disorder. Positive results in this screening assay, when used for newborn screening, are confirmed with standard PCR and Southern blot analyses.

Southern blot analysis is the second method of testing, which provides a rough estimate of repeat size for each allele by using restriction enzymes that are sensitive to the DNA methylation status at a recognition site near the expansion. This test can distinguish between normal, intermediate, premutation, and full mutations, but precise determination of number of repeats is not possible. This test is most useful if a full mutation is suspected or to verify that a female appearing homozygous for a repeat size on PCR is not harboring a large repeat expansion unable to be detected by PCR. To distinguish precisely between intermediate size alleles and premutations, PCR analysis is the preferred method. Southern blot also allows for determination of methylation status. Larger quantities of DNA are required for Southern blot than for PCR and the turnaround time is also longer.

Individuals who received cytogenetic testing or chromosome linkage analysis in the past for fragile X should be offered genetic testing using current technology to

verify the previous results. The website www.genereviews.org provides a full listing of laboratories that offer FMR1 analysis.

Though we discuss FMR1 allele length in terms of CCG repeats, there are interruptions in these long tracks of CGG repeats. These interruptions contain a single AGG repeat usually in the, 10th, 11th, 20th, or 21st spot in the FMR1 allele. These interruptions act as a sort of "fence post" in the long CGG repeated allele (see Chap. 6). The recent literature supports the use of AGG interruption analysis to determine the risk of expansion in premutation carriers. Previous methods of determining this risk were based solely on the number of CGG repeats and the gender of the carrier. However, premutation allele stability is also related to the number of interspersed AGG interruptions. Analysis for AGG interruptions is now available through several laboratories. This analysis is most helpful for premutation carriers who have no family history of individuals with a full mutation to determine the chance of expansion in a current or future pregnancy. AGG interruption analysis is also helpful to determine the instability of the intermediate allele in ascertaining the risk for expansion in the next generation.

Genetic Counseling

Genetic counseling is defined by the National Society of Genetic Counselors (NSGC) as the process of "helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease." This process includes interpretation of family and medical histories to assess the chance of disease occurrence or recurrence, education about inheritance, testing, management, prevention, resources and research, and counseling to promote informed choices and adaptation to the risk or condition (NSGC 2005).

Due to the multigenerational nature of FMR1 mutations and the implications for extended family members along both horizontal and vertical lines of the family pedigree, the genetic counseling process is complex and can require multiple sessions with various members of the family. Genetic counseling for fragile X-associated disorders is complicated by the variable phenotypes of the pre- and full mutations and the implications for family members. In order for the information to be appropriately integrated and communicated, the emotional, psychosocial, and cognitive status of individuals learning about the implications of FMR1 mutations should be considered. As with all genetic counseling sessions, strategies, suggestions, and development of a plan to assist the family in informing other family members about their risk should be included.

Genetic counseling sessions begin by obtaining the medical and psychosocial history from the patient and/or family members. A detailed family history (at least three generations) must be obtained and analyzed. It is often through the use of the family history that a counselor is able to gain more information about the patient and assess which family members may also be at risk for FXD. There are specific questions which should be asked when obtaining the family history.

When eliciting a family history, a genetic counselor will start with the proband, or first identified family member with the FMR1 gene change. The proband may be an individual who has been diagnosed as having FXS and thus brought attention to the family member with suspected or diagnosed FXTAS. However, the proband may also be a patient with FXTAS, FXPOI, or another carrier as the first presenting family member.

If the family member who has FXTAS is the proband, then the genetic counselor will work forward through descendants or offspring, backward through parental and grandparental generations, and horizontally through the siblings of the proband. The counselor will want to identify all members of the family who have been identified by previous testing or are suspected by family order or history to be carrying the FMR1 mutation.

When providing genetic counseling for FXD, it is important to be global and comprehensive in thinking. In the past, intellectual disability was the only criteria used to determine if a family member had FXS or was at risk to have a child with FXS. Now, a genetic counselor must consider not only intellectual disability but also autism spectrum disorders, FXPOI, immune-mediated disorders, movement disorders, and dementia to assess the risk for an FMR1 mutation. As clinical features of FXTAS are more identifiable, the number of probands who present with FXTAS is increasing and often there is no known history of FXS, autism, or other learning disabilities in the family. Therefore, when a genetic counselor encounters a patient with FXTAS or suspected FXTAS, it is important not only to ask questions related to FXTAS itself but also to probe with questions related to emotional, psychiatric and reproductive history, learning patterns, educational history, immune disorders, as well as age and cause of death for biological relatives (Hagerman et al. 2008). Specific questions for any female family member at risk to carry the premutation should include a history of or current anxiety (generalized or social), depression, mood lability, panic attacks, obsessive compulsive disorder, primary ovarian insufficiency (FXPOI), and immune-mediated disorders including thyroid disorders (Coffey et al. 2008; Wheeler et al. 2014). Questions for any male member at risk to carry the full mutation should include a history of intellectual disability, autism or autism spectrum disorder, ADHD, learning problems particularly in math, anxiety, depression, shyness, gaze avoidance, hand flapping, perseveration, sensory issues, reflux, ear infections, and epilepsy (Clifford et al. 2007; Cornish et al. 2004). The previous questions would also apply to a female suspected to have the full mutation including the addition of shyness, selective mutism, impulsivity, or social immaturity (Keysor and Mazzocco 2002).

Because of the wide range of phenotypic effects associated with *FMR1* expansions, both in terms of severity and age of onset, fragile X mutation testing in asymptomatic minors warrants special consideration. When the minor relative of an individual with a known *FMR1* mutation exhibits behavioral, developmental, and/or relevant medical symptoms (e.g., abnormal menses), and if the pedigree is consistent with fragile X inheritance, *FMR1* mutation analysis may be considered a diagnostic study. In cases where a minor is completely asymptomatic, testing is not so straightforward. Several professional organizations have published position statements urging caution when considering carrier and/or presymptomatic testing

for minors (American Academy of Pediatrics et al. 2013; Finucane et al. 2012; Ross et al. 2013). Genetic counseling in such cases is strongly recommended, and testing should be offered on a case-by-case basis.

There are standard *FMR1* genetic counseling practice recommendations available at the National Society of Genetic Counselors Web site, www.NSGC.org, to assist with the genetic counseling session. To locate a genetic counselor within the geographic area of the patient, there is a "find a counselor" feature on this same website. The patient's zip code is necessary to use this feature. You can also use the feature to refer an out-of-the-area family member to a local genetic counselor in their area.

Reproductive Issues

Adult Male Carriers

In most cases, the adult male diagnosed with FXTAS will have completed his reproductive years. Male fertility is not affected by the FMR1 premutation. However, in the event a male with FXTAS, or at risk for FXTAS, is considering having offspring, or that his daughters (who are all obligate carriers) are engaged in the genetic counseling process, reproductive options should be addressed in the genetic counseling session.

Prenatal diagnosis or other assisted reproductive technologies are increasingly being requested by male carriers. For these individuals, options do exist to determine the fetal gender and genetic status. Sperm sorting to reduce the chance of having a carrier daughter may be pursued though sperm sorting but this is by no means a highly accurate process, usually tilting the 50/50 male:female ratio to approximately 60–80/40–20 with no guarantees of one gender or the other (Cran and Johnson 1996). Prenatal sex determination by ultrasound is usually obtainable in the second trimester and can inform the parents of the gender. However, to determine the actual CGG repeat number, the pregnant partner would have to undergo prenatal diagnosis such as chorionic villus sampling (CVS) recommended to be performed 10.5–11.5 weeks gestation (Castellvi-Bel et al. 1995) or amniocentesis at 15 weeks or later.

Other options for couples in which the male is an FMR1 premutation carrier include adoption or using a noncarrier sperm donor to eliminate the chance of a carrier daughter. In this case, it is important to have the sperm donor tested to rule out his being a premutation carrier (Wirojanan et al. 2008).

Prenatally Detected Male Carriers

When prenatal diagnosis is requested for fragile X, it is usually to determine whether or not the fetus has a full mutation and will/may be affected with FXS. However, if the male fetus is found to have a premutation, the genetic counseling session may include the risk for FXTAS in adulthood as well as the risk (although small) for the

child to have cognitive, autism spectrum, and/or ADHD symptoms, if the premutation is large (e.g., 150–200 repeats) (Farzin et al. 2006). Depending on the needs of the couple, this discussion may be brief or extensive and has the potential to increase some anxiety about the child's future. Other issues, such as when to tell the child about their genetic predisposition, and concerns related to "the crystal ball" effect of knowing about a risk before symptoms, may emerge.

Genetic counseling for couples for whom a male carrier has been detected by prenatal diagnosis is particularly complicated because of the future risk for FXTAS. Some may consider the prenatal detection of male carriers to fall under the guise of presymptomatic testing, such as seen in some adult onset diseases. Genetic counseling sessions with parents of prenatally detected carrier males should be considered with an appreciation of the ethical dilemmas that presymptomatic testing provokes. Potential dilemmas include the inability of the child to give informed consent for testing regarding an adult onset condition, and the additional anxiety that may be placed on and internalized by the child as he grows up with the knowledge that he is at risk for this late onset condition (De Jong and De Wert 2002). However, as knowledge about the premutation increases, these concerns or risks need to be weighed against the benefits of knowing the emerging clinical picture and staying abreast of new developments.

Genetic Counseling for *FMR1* Premutations in Pregnancy

With widespread adoption of *FMR1* population screening in pregnant women, the number of couples receiving an unanticipated fragile X diagnosis during pregnancy has increased. The most common abnormal result on maternal carrier testing involves an intermediate allele which requires genetic counseling but poses minimal risk for an adverse pregnancy outcome. By contrast, the finding of a maternal *FMR1* premutation during pregnancy has important implications for the woman herself, her unborn child, and her extended family. These multiple levels of impact, in addition to the complex inheritance pattern of fragile X-related disorders, can cause significant stress for newly identified pregnant couples and unique challenges for genetic counselors and health care providers.

The primary concern for pregnant couples is usually focused on the risk for fragile X syndrome in the current pregnancy. With increasing awareness of non-invasive prenatal testing (NIPT) for Down syndrome and other conditions, it is now commonplace for pregnant women to inquire about its availability for fragile X syndrome. If the identification of a maternal premutation occurs early in pregnancy, a couple may opt for prenatal diagnosis. Some couples will not consider invasive prenatal diagnostic procedures but may choose maternal AGG analysis to get a more accurate assessment of risk.

Most couples experience relief when learning that a woman's premutation has not expanded to a full mutation in an unborn child. This can deflate concerns about premutation-related disorders, yet there are significant considerations that should be discussed. Because FXTAS is an adult onset disorder, it is often, but not

always, viewed by couples as a potential long-term health condition that can be acknowledged and dealt with in the future. Genetic counseling related to an unborn child's future FXTAS risk is similar to that for other late-onset conditions, such as familial Alzheimer's disease, that have their onset in the fifth decade or later. Likewise, the chance for FXPOI in a female should be discussed. While the chance for neurodevelopmental and psychiatric symptoms due to *FMR1* premutations is relatively low, the potential for an adverse outcome still exists and appears to be somewhat higher than chance. This small, unquantified increased risk for an unborn child with a premutation should be discussed in the broader context of the general population risk for neurodevelopmental disorders in all children, regardless of *FMR1* status.

Reproductive Options for Female FMR1 Carriers

Most females diagnosed with FXTAS will also be beyond their reproductive years, but they may have daughters who are also carriers of the premutation. Since all of the daughters of male carriers will be obligate carriers, they should be offered genetic counseling regarding their risk to have offspring with FXS. The reproductive options for FMR1 female carriers are dependent on the fertility status of the carrier. Some of the assisted reproductive technologies will require the carrier to have adequate ovarian function for such procedures. The reproductive options for female carriers include the following:

- Achieve pregnancy and forego any prenatal testing
- Prenatal testing (see below)
- Egg donor or embryo transfer
- Preimplantation genetic diagnosis (PGD) (see below)
- Adoption
- Choose to not have any (or additional) children

Prenatal Diagnosis

CVS and amniocentesis are available to determine the genetic status of the fetus. If a woman is having a procedure for maternal age or any other risk factor that does not include fragile X, the tissue or fluid is *not* routinely analyzed for the FMR1 gene mutation unless FMR1 gene analysis is specifically requested.

CVS involves taking a small sample from the developing placenta. The placenta contains genetic material that is of fetal origin. The cells can be studied for chromosome abnormalities and for the FMR1 gene mutation, when requested. CVS is performed earlier in the pregnancy than amniocentesis, and for detection of the FMR1 gene mutation it is suggested that the sample be taken between 10.5 and 11.5 weeks

of gestation. CVS does not give information about methylation. However, many couples will prefer to have the diagnosis made earlier and by repeat number. Like amniocentesis, the risk for miscarriage is small (less than 1 %).

Amniocentesis uses a small (two tablespoons or more when FMR1 gene analysis is performed) sample of the amniotic fluid. This fluid has fetal cells that can be grown and studied for various genetic conditions. The procedure is performed no sooner than 15 weeks gestation for FMR1 analysis and results can take up to 3 weeks. Methyation status can be determined by amniocentesis. Noninvasive prenatal testing is not applicable to FMR1 testing.

Some families will consider prenatal testing to be an option only for those who would consider termination of a pregnancy, should the FMR1 test be positive. As a genetic counselor presenting reproductive choices or working with a pregnant premutation carrier, it is important to discuss the option of prenatal diagnosis as a way to obtain information about the fetus and enable the family to make decisions. If pregnancy termination is not being considered, there may be benefits to prenatal testing. If the testing is negative, the patient can continue the pregnancy without anxiety as to whether or not the fetus has FXS. If the testing is positive, the parents may proceed with their choice of pregnancy termination or have time to adjust, grieve, and prepare for a child who will have FXS. The parents will also have time to speak with professionals prior to delivery and make choices around the medical and therapeutic interventions that can begin as early as possible in order to maximize the development of the child. However, for some couples prenatal testing, and its inherent small miscarriage risk, is not an option.

Egg Donation

Another option for female carriers of the premutation is the option of conceiving using eggs donated from a non-FMR1 mutation carrier. The benefits of this option are that it does not require the carrier to have an adequate egg supply of her own, and it eliminates the risk for FXS as long as the donor is negative for the gene mutation. Though it is costly, because it involves in vitro fertilization, it is not as expensive as preimplantation genetic diagnosis (PGD). It is important to have the egg donor tested to rule her out as a carrier of the FMR1 gene mutation. However, if a couple wishes to have a child who is biologically related to the mother as well as the father, the option of egg donation may not meet their needs.

Embryo Transfer

Embryo transfer is another option that can be offered to couples who are at risk to have a child with FXS. This option involves the use of a fertilized embryo that is biologically unrelated to either parent. This option is also expensive because it too involves in vitro fertilization but is less expensive than PGD.

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis is a process whereby a woman achieves a pregnancy by in vitro fertilization (IVF) with her own unaffected embryo. Unfertilized eggs are removed from a woman's ovary and sent to a laboratory experienced in PGD for the FMR1 gene mutation. The eggs are fertilized each by a single sperm, and then the embryo is tested for the FMR1 mutation.

PGD methods for fragile X include blastomere biopsy (embryo biopsy) and polar body analysis which are initially performed on the first polar body produced from the division of the egg. The polar body can be removed and analyzed to see if it carries the FMR1 gene mutation. After fertilization the egg undergoes another cell division which produces the pre-embryo and the second polar body. The second polar body is then tested and when these results are combined with the results from the first polar body test and the maternal genetic markers, the maternal genetic contribution in the embryo can be determined. Some PGD labs use only the polar body analysis, some use the blastomere biopsy, and some use both.

For blastomere biopsy, one or two cells of the embryo are tested when the entire embryo is at the undifferentiated six- to eight-cell stage. Results from testing are available approximately 3 days after retrieval. Only those embryos without the mutation, as determined by polar body analysis and/or blastomere biopsy, are used to generate embryos for implantation into the woman's womb.

One of the challenges in performing PGD for female FMR1 carriers is that many carriers experience ovarian insufficiency (ranging from premature menopause to reduced ovarian functioning) during the reproductive years, making it difficult to hormonally stimulate the ovaries to produce the multiple viable eggs needed for PGD (Coffey et al. 2008; Mila and Mallolas 2001). Another challenge is the difficulty of testing for the FMR1 gene mutation in the PGD setting. The PCR technology used in PGD requires that the genetic status of the embryo be determined based on analysis of one or two cells.

There are two stages to this process. The in vitro fertilization (IVF), available at many infertility centers, withdraws and then implants the fertilized egg. The PGD, i.e., the genetic testing of the egg for the gene mutation, is done separately and only at a small number of PGD laboratories in the world. When undergoing PGD, the carrier may have her IVF at a center close to home or at the center performing the PGD analysis. Prenatal testing is then recommended at the appropriate stage of gestation in order to verify the results of the PGD molecular testing.

The fees for PGD will depend on the number of cycles, distance from the PGD center, other testing to be performed (such as chromosome analysis), medications, etc. Health insurance may cover a portion of the cost. The advantage of PGD is that it is often an acceptable option for those who would not consider a pregnancy termination or want to have a child without FXS and it allows for the possibility of having a child who is biologically related to both parents. The disadvantages are discussed above (Geraedts et al. 2001).

Special Considerations in Genetic Counseling for FXTAS

> I am the caregiver to one who is never mean spirited, impatient, nor unkind. He is still fun to be with, to laugh with, and to enjoy what we can together. I am 'blest'! And at the end of the day, we 'scoop each other up, hold each other tight and tell each other everything is all right.'
>
> —Dorothy and John Kinna

Genetic counseling for FXTAS is complicated because in addition to determining the neuropsychiatric features, sharing genetic information, and obtaining the family pedigree, there are many psychosocial issues to address (see Chap. 3) (Bourgeois et al. 2006; Grigsby et al. 2006). These psychosocial issues include not only the FXTAS patient but also his/her caregiver, which in most cases is the spouse.

The psychosocial issues related to FXTAS include understanding of the disorder, the integration of this understanding into the patient's self-image, current and future manifestations of the disorder, prognosis, treatment and interventions, and long-term planning. Complicating understanding of these issues are the cognitive and psychological disabilities that may be present in the patient with FXTAS (Hessl et al. 2005).

The FXTAS patient can be overwhelmed with too much information and with the recommendations that affect their quality of life, such as the cessation of driving or need for a cane, walker, or wheelchair. Impotency and incontinence also have the potential to threaten self-esteem and so need to be addressed with sensitivity. In addition, the patient may not be aware of the severity of his/her FXTAS-related clinical findings and find it depressing and threatening to have these addressed.

> My husband's condition started with shaking hands, making it difficult to write, then deteriorating to not being able to write and almost impossible to hold a glass without spilling the contents. Then his gait was unsteady. He started using a cane, then a walker and eventually a wheelchair. For a long time, we were uncertain of the diagnosis or what to expect. Then our grandson was diagnosed with FX and we met the staff at the M.I.N.D. Institute.
>
> —Marilyn Darwin, wife of Richard (deceased)

The FXTAS patient cannot be treated in isolation. The questions, concerns, and needs of the caregiver/spouse must also be addressed as these individuals experience varying degrees of stress (Bacalman et al. 2006; Gane et al. 2005, 2010; Seritan et al. 2008). As in Alzheimer disease and other neurological disorders, it is important to recognize that the caregiver may be prone to depression, feelings of being overwhelmed and isolated, increased fatigue, grief over the loss of a planned future, anger regarding the patient's irritability and lack of support, and a lack of understanding of what the future needs of the patient may be (Gane et al. 2005). It is important to encourage the caregiver to maintain their own network of friends and activities outside of the home so that they develop independence and an identity beyond that of "caregiver."

> We had wonderful support from our family, friends and church. There were always people coming to visit, bringing something appetizing to eat or to run an errand for us. The last 6 months of his life, hospice was a great support to both of us. We felt it was important to keep

> us both involved and relating to people. For me, an active support group is a God-send in the final transition in life.
>
> —Marilyn Darwin, wife of Richard (deceased)

Additionally, the issues of long-term care and death must be addressed with the caregiver. They are often not addressed by the primary care provider or other health professionals, and this information may be used in family financial planning, in addressing accommodation and physical support needs, exploring future care options, making choices regarding a living will and/or autopsy wishes, and planning for life alone.

FXTAS is a unique entity and as such requires a creative model for genetic counseling. The counseling session needs to be designed not only to adequately address the genetic aspects of the FMR1 gene, FXD, and inheritance patterns but also to meet complicated medical, psychosocial, and emotional needs. A model that can be used is one in which the counselor meets with both members of the couple together for a short period, then meets with each of the couple individually, and ending by bringing the couple back together again to counsel and summarize their individual understanding of the issues that have been addressed and to help them to be supportive of each other. A verbal consent should be gained and documented from each of the couple for issues that have been discussed in the individual session and are to be raised again when they are together. Using this model, the counselor can obtain a more accurate understanding of the patient's progression and current clinical findings as well as encourage intimacy and sensitivity as the couple work in partnership to address FXTAS-related issues. Monitoring body language and wording provides insight when conveying information to the couple as well as interpreting how the information is being received.

> We truly meant it when we looked into the future 49 years ago and said 'for better, for worse, in sickness and in health, 'til death do us part.'
>
> —Dorothy and John Kinna

Psychosocial Issues

Genetic counseling for FXTAS requires an awareness of psychosocial interactions between the counselor and the patient, as well as patient and spouse/caregiver and spouse/caregiver and counselor. For the purposes of this section, we are assuming that the caregiver is the spouse of the person diagnosed as having FXTAS since this is most frequently the case.

The patient and spouse will often have a different perception of the physical, cognitive, and emotional changes that have already occurred and may occur in the future for the patient. In addition, both will have different psychosocial and emotional issues that need addressing at the time of the genetic counseling session (Gane et al. 2005). The counselor may become aware that more than one session may be necessary to provide the appropriate care and guidance. As in all counseling sessions, the ethical issues of patient autonomy, confidentiality, and beneficence must be respected.

Understanding the clinical symptomatology of FXTAS is also an important requirement when providing counseling to this population. In the male patient, his physical, cognitive, and emotional needs can infringe upon the session. These may include the necessity for frequent breaks due to fatigue, toileting frequency and incontinence, as well as loss of concentration and the inability to follow the information being shared. The counselor needs to be aware that irritability, mood lability and disinhibition, as well as other signs of frontal and executive functioning deficits, can impair the flow of the counseling session necessitating the counselor's flexibility in how information is presented and how the needs and concerns of the patient and spouse are addressed. The frontal and executive function deficits are often manifested in the patient's reception and perception of information shared by the counselor. This can be seen when such issues as employment, the ability to drive and to make life decisions, disease progression, need for walking devices, and health issues are discussed. Additionally the emotional ramifications of FXTAS such as denial, anger, grief, loss of self-esteem and self-worth can impact the counseling session. Often the male patient is not truly aware of the impact of FXTAS on his current level of functioning, understanding and his ability to communicate. The counselor should assess these issues as he/she seeks to help the patient and spouse comprehend the current status of the patient and prepare for the future as FXTAS progresses.

When the patient with FXTAS is a female, the counseling session can include other issues as well. Understanding and defining the clinical presentation of FXTAS in a female patient is often challenging because the features are still being defined and can be ameliorated by the presence of the second X chromosome carrying a typical number of repeats. In this situation, it is recommended that the genetic counselor determine that a physician knowledgeable about FXTAS has made the diagnosis and that information pertaining to the specific clinical features of the patient be addressed by the counselor. The clinical features can include tremor, ataxia, cognitive changes, and autoimmune disorders (Coffey et al. 2008). The severity of the clinical presentation will determine the pace and needs of the genetic counseling session as well as the physical and emotional concerns of the patient and her spouse/caregiver.

Since FXTAS is a progressive disorder in males and in some females, it is not uncommon for the genetic counselor to have to work with a patient and spouse who are grieving, angry, or in denial about the course and prognosis of the condition.

> I spent many hours crying my heart out and also being very angry… I felt trapped … I felt like running screaming out of the house.
>
> —Terri Corcoran

It is not until the patient and spouse meet with a medical professional knowledgeable about FXTAS that the couple may begin to come to terms with the diagnosis. The counselor needs to be aware of whether or not the clinical features of FXTAS have been diagnosed accurately in the male patient, or considered to be signs of hysteria, imagined, or overreported in the female patient. The incorrect diagnosis can cause frustration and confusion for the patient and family members. Prior to the correct diagnosis being made, many of the features of FXTAS in the

male patient may have been explained away by the family as simply aging, especially those features pertaining to the cognitive changes (Jacquemont et al. 2004b). For the female patient, the family may have been dismissive and labeled her as being a hyperchondriac. When an incorrect diagnosis has been made, the counselor must help the patient and family members to understand the true underlying medical cause of the patient's condition, to validate their emotional response to the change in diagnosis, and help them to adapt to the new diagnosis.

Currently, depression in the spouse seems to be the primary finding. However, depression can be the outward sign of anger due to the demands of caregiving as well as anger and grief due to the loss of a future that has been planned for the retirement years. Additionally, as FXTAS progresses, it often manifests with mood changes and increased irritability directed toward the spouse. Too often, the counselor will hear the spouse say, "He is not the person he used to be." The individual who was once patient and forbearing can become impatient, seem to be irrationally angry, and may not want their wife to leave them alone. As the symptoms increase, they lead to greater physical burden being placed upon the spouse such as weight bearing, lifting, cleaning, dressing and bathing. A recommendation to a physician for evaluation of antidepressant medication as well as a recommendation for professional counseling from a psychologist, licensed social worker, or family therapist may be necessary in addition to addressing respite care needs.

> The first years of our marriage were extremely difficult. I struggled with convincing Vince to retire from his business… I struggled with repairing his house… I struggled with all the financial catastrophes he had gotten into as his mind was failing; I struggled with adjusting to his onslaught of disabilities, buying walkers, transport chair, building a wheelchair ramp, installing stair lifts. I struggled with helping him walk, shower, dress, shave, brush his teeth. I struggled with getting him up when he fell … calling the rescue squad or looking for neighbors to help when I couldn't lift him up any more. Even more difficult for me were the emotional struggles.
>
> —Terri Corcoran

End-of-Life Planning

At the appropriate time during the genetic counseling session, it may be necessary to discuss end-of-life planning. Family members will often ask for information regarding planning in this context. The patient with FXTAS and/or spouse may voice a need to help their grandchild with FXS or others with FXTAS through such planning. One way to address this issue is to offer with sensitivity the opportunity for multiple tissue and organ donation after death. Several groups in the United States are using tissue donation to learn more about FXTAS, the FMR1 gene, and mRNA function and structure. For more information, contact the following groups: The M.I.N.D Institute, Sacramento, CA; Maryland Brain Bank, Baltimore, MD; and Emory University, Atlanta, GA.

Financial planning for the spouse after the death of the patient with FXTAS is another issue that may arise during the counseling session. It is not uncommon for

the male patient with FXTAS to express concern regarding financial planning for his spouse and for any grandchildren with FXS. The genetic counselor should be aware of how pertinent this area is for the family and be able to validate these concerns if voiced and to make the appropriate referrals.

It is important that the genetic counselor or another professional be able to address the various issues related to end-of-life planning and care. The patient and/or spouse will be more comfortable voicing questions and concerns around these issues if the professional is comfortable in discussing these areas and able to offer supportive planning as well as emotional support.

Genetic Discrimination

The patient who has been diagnosed with FXTAS may express a concern about genetic discrimination, the misuse of an individual's genetic test results, or knowledge of that individual's family history of a hereditary condition resulting in differential treatment. This concern may prevent some patients from choosing to undergo genetic testing or clinical evaluation for a genetic condition such as FXTAS. Individuals may fear that knowledge of their genetic risk for a possible future condition may cause them to lose their insurance, job or adversely affect their career. These are valid considerations, as often the patient with FXTAS is already being faced with these consequences as a result of his/her medical condition. There may also be concern that health, life, disability, and long-term insurance companies may obtain results and use this information to deny some forms of coverage or raise insurance premiums. Genetic discrimination has the potential to create significant social and financial burdens for individuals with a family history of a hereditary condition. Patients with FXTAS and/or their spouses may voice these concerns.

Parents of children with FXS are concerned about discrimination in the context of having a child with special needs, but one study found that there is more concern than there are actual reports of discrimination by insurance companies among these families (Wingrove et al. 1996). However, discrimination by single carrier insurance plans asked to cover individuals with FXS has been reported to the author. There are no published reports or studies on the occurrence of insurance discrimination for individuals with the FMR1 premutation or with FXTAS.

The experiences of asymptomatic individuals with a family history of Huntington disease, another adult onset neurodegenerative disorder, show that discrimination does occur and can include an increase in insurance premiums, the inability to obtain insurance coverage, and forced retirement based on genetic test results and/or knowledge of the family history (Bombard et al. 2008). There are no reports, however, showing that this has been the experience of those with fragile X-associated disorders such as FXTAS or those carrying the FMR1 gene mutation.

State laws vary widely in the level of protection against the use of genetic test results in determining insurance eligibility and employment. The Americans with

Disabilities Act, Title VII of the Civil Rights Act of 1963, Executive Order 13145, and the Health Insurance Portability and Accountability Act offer some protection. However, there are some areas of protection that are lacking such as individual health plans and use of predictive genetic test results. The Genetic Information Nondiscrimination Act of 2008 (GINA) is a federal law that prohibits the use of predictive genetic test results, i.e., DNA and RNA, for asymptomatic individuals in setting premiums and determining eligibility for health insurance and employment status. It is too early to determine the impact of this law on any specific genetic condition. More work is needed to prevent genetic discrimination as current state and federal laws do not offer full protection. However, with the Affordable Health Care Act, these concerns may be addressed.

Treatment Issues

Counseling about the future of FXTAS should include a focus on treatment and the genetic counselor may need to discuss the current treatment options with the patient. These treatment options include specific medical and therapeutic interventions (see Chap. 9). Additionally, cognitive behavioral therapy (CBT), specifically problem solving therapy (PST), is now being explored as a therapeutic treatment to address the executive function deficits and depression documented in the FXTAS patient and his/her spouse in order to facilitate the development of healthier coping strategies (Alexopoulos et al. 2003; Bacalman et al. 2006). Cognitive behavioral therapy is a short-term psychotherapeutic approach that has been used effectively to treat the emotional and psychosocial problems of patients who have Alzheimer disease and their caretakers (Seritan et al. 2008) and is expected to be helpful for the FXTAS population.

Summary

The components of genetic counseling for FXTAS and fragile X-associated disorders continue to evolve. Although the fundamentals of the genetic counseling session itself provide a foundation within which the information pertaining to FXTAS and FXD can be shared between the counselor and the patient, the amount of information that is involved continues to grow and fundamental understanding continues to be clarified. FXTAS exemplifies this ongoing evolution and thus provides unique challenges for the counselor. Therefore, when meeting with a patient who has FXTAS, it is important that the genetic counselor turn to outside resources in order to provide up-to-date information as well as support to the patient and his/her spouse. A primary resource is the National Fragile X Foundation (NFXF) at www.fragilex.org. The NFXF website also contains a list of the clinics that specialize in FXTAS, FXS, and other fragile X-associated disorders, which can be accessed by

professionals, patients, and family members. Information can also be obtained from the FRAXA Research Foundation at FRAXA.org.

> As profoundly as FXTAS has negatively impacted our lives and marriage, I have gained some profound positives. I have an ever-growing faith in a God who loves and guides me. I have learned to view life from a more mature perspective. I have become a much more compassionate person. I have learned to appreciate very simple things that can bring a peaceful interval in the midst of catastrophic illness. Most importantly, I really believe that Vince does love me. Every so often he tells me so. I am very lucky that Vince is content with his quiet life. He never complains and hasn't had any angry outbursts for years. I derive great peace and comfort just from being beside him and drawing strength from his quiet presence.
>
> —Terri Corcoran

Acknowledgments We would like to thank all the patients who have been diagnosed with FXTAS, FXD, and their many family members including those who have made contributions to this chapter. It is because of each of them that we continue to learn, change, and grow in our understanding of fragile X-associated disorders.

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