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Review

Fragile X syndrome and associated disorders: Clinical aspects and pathology

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ABSTRACT

This review aims to assemble many years of research and clinical experience in the fields of neurodevelopment and neuroscience to present an up-to-date understanding of the clinical presentation, molecular and brain pathology associated with Fragile X syndrome, a neurodevelopmental condition that develops with the full mutation of the *FMR1* gene, located in the q27.3 loci of the X chromosome, and Fragile X-associated tremor/ataxia syndrome a neurodegenerative disease experienced by aging premutation carriers of the *FMR1* gene. It is important to understand that these two syndromes have a very distinct clinical and pathological presentation while sharing the same origin: the mutation of the *FMR1* gene; revealing the complexity of expansion genetics.

1. Introduction

The expansion of the trinucleotide CGG above normal range (> 54 repeats) in the non-coding region of the Fragile X Mental Retardation 1 (*FMR1)* gene (Fig. 1) is responsible for the development of the fragile X-associated disorders in those carrying the premutation (55–200 CGG repeats), including fragile-X associated tremor/ataxia syndrome (FXTAS)(Hagerman et al., 2001; Jacquemont et al., 2003), fragile X-associated primary ovarian insufficiency (FXPOI) (Sherman, 2000) and fragile X-associated neuropsychiatric disorders (FXAND)(Hagerman et al., 2018); and the presence of fragile X syndrome (FXS) in those carrying the full mutation (> 200 CGG repeats). This review details the clinical presentation and neuropathology of the two entities affecting normal brain function: FXTAS, a neurodegenerative disease that commonly develops during the seventh decade of life in 40% of premutation male carriers and 16% of female carriers (Hagerman and Hagerman, 2016); and FXS, a neurodevelopmental disorder found in 1:7000 males and 1:11000 females (Hunter et al., 2014) causing intellectual disability and Autism Spectrum Disorder (ASD) in more than half of those affected (Hagerman et al., 2017).

2. Fragile X syndrome

2.1. Clinical aspects

FXS is caused by the lack or deficiency of the *FMR1* protein FMRP in both males and females with a full mutation. With CGG repeats of > 200 there is typically silencing of *FMR1* through methylation. The subsequent lack of FMRP, a regulator of translation, leads to dysregulation of hundreds of proteins that affect synaptic plasticity and connectivity in the developing brain leading to intellectual disability (ID) and other clinical features of the syndrome (Danesi et al., 2018; Gatto et al., 2014; Higashimori et al., 2013; Wang et al., 2004; Pilaz et al., 2016). Along with the variable presentation of ID, 60% of boys and 20% of girls with FXS are also diagnosed with ASD (Bailey Jr. et al., 2008). The complexity of the clinical presentation is accentuated with a well reported psychiatric profile including general anxiety, social avoidance and hyperactive behaviors. These characteristics are commonly seen in those with and without the comorbid presentation of FXS and ASD (Bailey Jr. et al., 2008; Thurman et al., 2014; McDuffie et al., 2015). Other comorbid conditions frequently diagnosed during childhood in FXS are seizures (Berry-Kravis et al., 2010), recurrent otitis media, strabismus and obesity (Hagerman and Hagerman, 2002). A Prader-Willi like phenotype, with obsessive/compulsive behaviors, delayed puberty, small genitalia, hyperphagia and lack of satiation after meals

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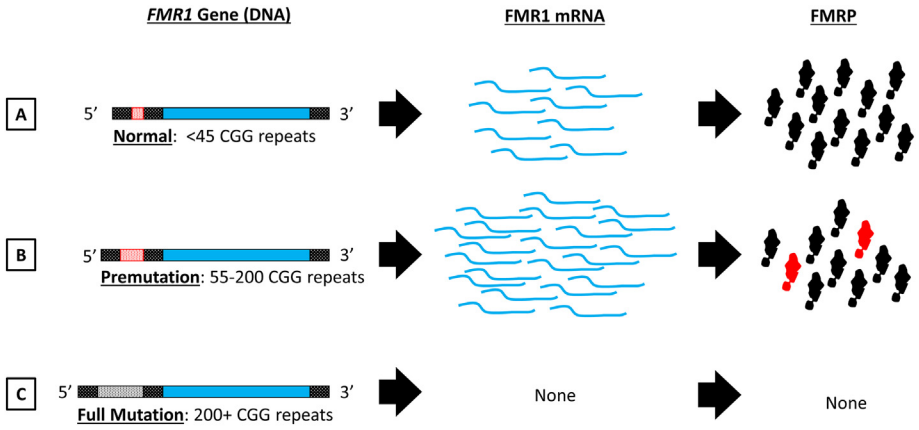

Fig. 1. *FMR1* GGG repeat length and gene expression. Overview of the relationship between CGG repeat length (A,B,C) within the *FMR1* gene (left column) and its effects on *FMR1* mRNA (middle) and FMRP protein synthesis (right). A) *FMR1* alleles bearing < 45 CGG repeats are considered in the normal range. B) CGG repeat expansion into the premutation range (containing 55–200 CGG repeats) causes an upregulation in FMR1 mRNA transcripts. For most premutation cases FMRP levels (black shapes) are not altered, although some individuals may show a modest reduction. Additionally, RAN translation of *FMR1* mRNA produces toxic FMRpolyG protein species (red shapes). C) CGG repeat expansion into the full mutation range (200+ repeats) causes hypermethylation of the *FMR1* gene, resulting in full transcriptional and translational silencing. Figure Key: *FMR1* Gene: Open reading frame indicated with solid blue, non-coding 5′ and 3′ regions indicated with shaded black pattern. CGG repeat is located in the 5′ untranslated region (red shaded in A and B, white shaded in C to represent hypermethylation of the gene). *FMR1* mRNA transcripts indicated with curved blue lines. FMRP protein represented as black shapes and FMRpolyG is represented as red shapes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leading to severe obesity, has also been described in < 10% of boys with FXS (McLennan et al., 2011; Nowicki et al., 2007; Fryns et al., 1987).

The physical characteristics of FXS include an elongated face, broad forehead, high palate, prominent ears, hyperextensible finger joints, flat feet and macroorchidism (during and after puberty) (Hagerman and Hagerman, 2002). However, classic facial characteristics have differences inherent to age and ethnicity (Lubala et al., 2018). In addition to commonly recognized characteristics, patients can present with a variable presentation of connective tissue alterations. Their presence is attributed to FMRP dysregulation of essential components of the extracellular matrix including elastin. Phenotypic findings related to connective problems include soft velvet-like skin, joint hyperextensibility, particularly in the fingers, double jointed thumbs, flat feet with pronation, mitral valve prolapse, dilated aortic root, and occasional scoliosis (Ramirez-Cheyne et al., 2018).

After puberty, there is a tendency for improvement of the most problematic behaviors during childhood, including aggression, hyperactivity and irritability; however, behavior and comorbidities can also worsen in those exposed to exogenous neurotoxins (Salcedo-Arellano et al., 2016). During adulthood, patients with FXS seem to have an increased risk of hypertension, obesity, gastrointestinal disorders, parkinsonism, mood disorders, anxiety and in some cases dementia (Sauna-Aho et al., 2018; Utari et al., 2010). However, patients with FXS have a normal life span. The female phenotype differs from the males since they have the benefit of an unaffected X chromosome. Their cognition includes 30% with an IQ < 70 (intellectual disability), 30% with an IQ in the borderline range (Kates et al., 1997; Reiss et al., 1994; Kooy et al., 1999; Ellegood et al., 2010; Lai et al., 2016; Hinton et al., 1991; Greco et al., 2011; Sabaratnam, 2000; Tassone et al., 2012a; Rodriguez-Revenga et al., 2009) and 30% with an IQ in the normal range (above 80), but anxiety and attentional problems can occur in all groups. (Hagerman et al., 2017).

2.2. Diagnostic criteria

The diagnosis of FXS can only be confirmed using genetic testing. *Southern blot* analysis reports an expansion of the CGG trinucleotide number > 200 repeats in the 5′ untranslated region in the *FMR1* gene located on the X chromosome. The result is a full methylation of the gene and its subsequent silencing (Hagerman and Hagerman, 2002). However, in some cases individuals can present with mosaicism, showing variability in CGG allele size and in methylation patterns within and between different cell lines. This particular genotype benefits the clinical phenotype by improving both the cognitive and behavioral profiles of males and female patients with FXS (Hunter et al., 2019).

2.3. Cell and molecular pathology

The origin of all changes that lead to the molecular, pathological and clinical symptoms shown by individuals with FXS is the loss of functional FMRP (Fig. 1C). While CGG expansion leading to hypermethylation and functional silencing of *FMR1* is by far the most common genetic cause of FXS, loss of a functional FMRP due deletions or point mutation can also occur (Quartier et al., 2017).

While FMRP expression is ubiquitous, it is expressed at highest levels in the brain and testes (Bakker et al., 2000; Devys et al., 1993). FMRP expression has been detected in neurons, astrocytes, microglia, and oligodendrocyte precursor cells (Wang et al., 2004; Pacey and Doering, 2007; Gholizadeh et al., 2015), and it is largely localized in the cytosol of neurons, in close association with ribosomes of the endoplasmic reticulum, and at high levels in dendritic spines (Feng et al., 1997). FMRP can also appear in cytoplasmic granules that are transported to dendrites, axons, and pre-synaptic terminals in some neurons (Akins et al., 2012; Antar et al., 2004; Antar et al., 2005; Antar et al., 2006; Price et al., 2006; Christie et al., 2009), enabling localized translation (Li et al., 2009; Banerjee et al., 2018; Li et al., 2001). FMRP granules are also present in axon growth cones during development, likely playing a role in axon guidance, circuit formation, and synaptogenesis (Antar et al., 2006; Christie et al., 2009; Li et al., 2009).

FMRP is an RNA binding protein that regulates translation of numerous associated mRNAs. FMRP is largely considered a translational repressor that suppresses translation initiation and elongation of nascent proteins (reviewed extensively in Darnell and Klann, 2013; Richter et al., 2015). FMRP also binds and regulates miRNA and miRNA machinery (Richter et al., 2015; Li et al., 2008), thus exerting translational control through a separate but complimentary molecular mechanism. Accordingly, due to a loss of FMRP mediated translational repression, there is a modest (10–20%) but functionally significant elevation in FMRP-regulated proteins in FXS patients and in *FMR1* KO mice (Richter et al., 2015).

Some key FMRP regulated mRNAs/proteins include second messenger proteins involved in $mGluR_1$ and $mGluR_5$ signal transduction (EIF4E and S6K), (Antar et al., 2004; Antar et al., 2005; Gkogkas et al., 2014), $GABA_A$ and $GABA_B$ receptor subunits (Braat and Kooy, 2015; D'Hulst et al., 2006; Wahlstrom-Helgren and Klyachko, 2015), numerous voltage gated ion channels (Brown et al., 2010; Ferron, 2016;

Ferron et al., 2014), Bone morphogenic protein receptor 2 (BMPR2) (Kashima et al., 2016), matrix metalloproteinase 9 (MMP9) (Sidhu et al., 2014), and amyloid precursor protein (APP) (Westmark et al., 2011; Pasciuto et al., 2015). Many of these affected mRNA/protein species play a direct role in synaptic transmission. FXS dendrites in the cortex and hippocampus show increased spine density and size, and reduced spine maturity (Antar et al., 2006; Irwin et al., 2001; Rudelli et al., 1985). FMRP mediated suppression of the FMPR2-Cofilin pathway is necessary for normal dendrite formation and maturation – and accordingly disruption in this pathway through a loss of FMRP contributes to dendritic abnormalities in FXS. Suppression of MMP9, which is upregulated in the *FMR1* KO mouse, normalizes dendritic spine morphology and synapse formation (Bilousova et al., 2009). Additionally, loss of FMRP leads to excess soluble APP levels, which also contributes to a lack of dendrite maturation (Pasciuto et al., 2015). Normalizing APP levels in *FMR1* KO mice rescues alterations in synaptic spines, LTP deficits, and reduces audiogenic seizures (Westmark et al., 2011).

The excitation/inhibition imbalance hypothesis has been proposed to explain how cellular and circuit-level alterations in excitatory/inhibitory signaling may lead to clinical symptomology in idiopathic ASD (Rubenstein and Merzenich, 2003). Given the tremendous overlap in FXS and ASD symptomology, as well as the high rates of co-diagnosis in patients with FXS, this hypothesis is believed to largely apply to FXS as well. One way in which excitation and inhibition balance may be disrupted in FXS is through dysregulation of glutamatergic and GABAergic signal transduction. FMRP binds and regulates second messenger proteins that mediate metabotropic glutamate receptor I family ($mGluR_1$ and $mGluR_5$) signal transduction. When FMRP is absent, there is increased phosphorylation of two such downstream effectors - eukaryotic translation initiation factor 4E (EIF4E) and ribosomal protein S6 kinase (S6K), which leads to excess translation of mRNAs that are typically bound and regulated by FMRP (Gkogkas et al., 2014; Sawicka et al., 2016; Hoeffer et al., 2012). There is an increase in synaptic long-term depression (LTD) in *FMR1* KO mice, which is believed to be related to dysregulation in $mGluR_1$ signaling. Another consequence of $mGluR_1$ signaling dysfunction in FXS is a reduction in inhibitory retrograde endocannabinoid signaling by $mGluR_1+$ dendrites (Hagerman et al., 2017; Busquets-Garcia et al., 2013), which likely leads to increased glutamatergic signaling from upstream pre-synaptic glutamatergic neurons and increased excitatory tone. Deficits in GABA signaling have also been characterized in the *FMR1* KO mouse, suggesting that a lack of inhibitory GABAergic tone could also lead to hyperexcitability in the FXS CNS.

2.4. Neuropathology

Structural MRI studies have identified a pattern of regional volume alterations in patients with FXS, characterized by an enlargement in the caudate nucleus (Gothelf et al., 2008; Reiss et al., 1995; Hallahan et al., 2011; Lee et al., 2007; Sandoval et al., 2018) and lateral ventricles (Gothelf et al., 2008; Reiss et al., 1995; Lee et al., 2007), and a reduction in cerebellar vermis (Gothelf et al., 2008; Lee et al., 2007; Mostofsky et al., 1998). Alterations in caudate and cerebellar vermis appear as early as one year of age (Hoeft et al., 2010), and persist into adulthood (Gothelf et al., 2008; Lee et al., 2007). There also appears to be a moderate and region-specific alteration in cortical lobe grey matter volume, with modest reductions in temporal (Gothelf et al., 2008; Sandoval et al., 2018) and frontal lobes (Gothelf et al., 2008; Hallahan et al., 2011), and a modest increase in the parietal (Gothelf et al., 2008; Hallahan et al., 2011) and occipital lobes (Gothelf et al., 2008; Hallahan et al., 2011). Although less consistent, volumetric reductions of amygdala (Gothelf et al., 2008; Kates et al., 1997) and enlargement of hippocampus (Reiss et al., 1994) have sometimes been observed. White matter volumetric alterations have also been detected, including increased white matter volume in the septal fornix (Sandoval et al., 2018), increased brainstem-hippocampus tract and cingulate-corpus callosum tract volume (Hallahan et al., 2011), and decreases in frontal lobe (Hallahan et al., 2011) and cerebellar white matter (Sandoval et al., 2018). Most human FXS structural abnormalities are not recapitulated in the *FMR1* KO mouse - striatal volume is unaltered (Kooy et al., 1999) or reduced (Ellegood et al., 2010; Lai et al., 2016), and there is no change in cerebellar vermis volume (Kooy et al., 1999; Ellegood et al., 2010; Lai et al., 2016) or cortical lobe volume (Kooy et al., 1999; Ellegood et al., 2010).

The neuropathological correlates of these structural abnormalities in the human FXS brain are poorly characterized – there only exist a handful of such studies and all typically have very small sample sized ($n \leq 3$ for all). The earliest and most well characterized finding demonstrated that there are alterations in dendrites and synaptic spines in the postmortem FXS brain. More specifically, FXS cortical tissue in the occipital and temporal cortices have more dendritic spines (Irwin et al., 2001), and these spines are longer and immature (Rudelli et al., 1985; Hinton et al., 1991). Ultrastructural analysis also shows a reduction in synaptic size at dendritic contacts (Rudelli et al., 1985). Cerebellar Purkinje cells are reduced in number (Greco et al., 2011; Sabaratnam, 2000) and in dendritic arbor complexity (Greco et al., 2011), and the hippocampal structure presents with restricted hyperplasia in the CA1 region (Greco et al., 2011). Structural and functional MRI studies have both been able to correlate abnormal activation patterns with specific symptom domains in FXS patients. For example, intellectual functioning, as indicated by IQ, is inversely correlated with caudate volume (Hallahan et al., 2011) and positively correlated with cerebellar vermis volume (Gothelf et al., 2008).

3. Fragile X-associated tremor/Ataxia syndrome

3.1. Clinical aspects

First described in a publication in 2001, fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease that primarily affects premutation carriers (55 to 200 CGG repeats) and clinically presents with the core features of intention tremor and/or cerebellar gait ataxia (Hagerman et al., 2001). While different in etiology, FXTAS like FXS is more common in males due to the X-linked etiology of the *FMR1* gene. It is estimated from one study in United States that 1 in every 403 men are *FMR1* premutation carriers and of those, 40% will be diagnosed with FXTAS by their seventh decade. In comparison, women premutation carriers have a prevalence of 1 in every 209 and only an estimated 16% chance of developing FXTAS due to their having one regular functioning X chromosome (Tassone et al., 2012a; Rodriguez-Revenga et al., 2009). It is worth noting that while premutation carrier women develop FXTAS at lower rates, they are at risk for fragile X-associated primary ovarian insufficiency (FXPOI), the most heritable form of premature menopause or early ovarian failure. In addition, female premutation carriers report higher rates of psychiatric symptoms including anxiety, ADHD, depression, insomnia, chronic fatigue, and chronic pain. Which fall under the umbrella term of FXAND (Hagerman et al., 2018). While not all premutation carriers go on to develop FXTAS the prevalence for FXTAS does increase in age with one study of premutation men showing 17% being affected at 50 years, 38% at 60 years, 47% at 70 years, and 75% at 80 years of age (Jacquemont et al., 2004)

The hallmark radiological sign of FXTAS is an increased signal on a T2 flair MRI sequence in cerebral white matter especially on the middle cerebellar peduncles (MCP) (Brunberg et al., 2002). This characteristic sign is not often seen in women; however, MRI scans of female FXTAS brains reveal increased signal in the splenium of the corpus callosum and in the pons (Hall et al., 2015). Moderate to severe cortical and general atrophy and increased ventricular volumes is seen in both genders. One study that analyzed 322 magnetic resonance imaging scans confirmed that overall brain and cerebellar volumes were

Table 1
Major and minor radiological, clinical, and neuropathological signs of FXTAS. The molecular criteria include an *FMR1* premutation. Adapted from Hall et al. (2014) and Berry-Kravis et al. (2007b).

	Major (signs/symptoms)	Minor (signs/symptoms)
Radiological	Radiological sign: T2 FLAIR MRI Middle Cerebellar Peduncle (MCP)	Radiological sign: T2 FLAIR MRI white matter lesions in cerebral white matter Radiological sign: T2 FLAIR MRI Moderate-to-severe generalized brain atrophy Radiological sign: T2 FLAIR MRI white matter hyperintensity in Splenium of Corpus Callosum (CCS)
Clinical Signs	Intention tremor Cerebellar ataxia	Parkinsonism (bradykinesia, shuffling gait, masked facies) Neuropathy Executive function and memory deficits
Neuropathological signs	Intranuclear inclusions in CNS and PNS	

statistically smaller in premutation carrier males with FXTAS as compared to premutation carrier males without FXTAS and controls (Wang et al., 2017). Another study in young asymptomatic premutation carriers found no differences in measures of executive function with aged matched controls however, the premutation carriers showed a significantly longer manual movement and reaction times. Suggesting that these cerebellar changes might underlie motor deceleration that occurs before symptoms are detected (Shickman et al., 2018).

3.2. Diagnostic criteria

After the *FMR1* mutation is confirmed as a premutation carrier status, diagnosis is often made after patients approach their physician with complaints of an action tremor and/or an increase in falls and unsteadiness or ataxia. A definite diagnosis of FXTAS is given if the *FMR1* premutation carrier presents with at least one major radiological sign (refer to Table 1) along with at least one major clinical symptom (Berry-Kravis et al., 2007a; Hall et al., 2014). Other frequently seen but more minor symptoms are parkinsonism (bradykinesia, muscle rigidity, masked facies and slowed speech) and cognitive decline in executive function and moderate to severe short-term deficits. Other comorbidities seen in premutation carriers with FXTAS include autonomic dysfunction, thyroid disease, peripheral neuropathy including symptoms of numbness, tingling and pain, fibromyalgia, migraines, hypertension, bradycardia, sleep apnea, and irritability or depression. (Hagerman and Hagerman, 2016).

Currently there is no cure for FXTAS and treatment is based on alleviation of symptoms, such as the use of primidone or beta blockers for the tremor and SSRIs for irritability or depression.

3.3. Molecular pathology

In contrast to *FMR1* full mutation, which leads to transcriptional silencing of *FMR1* mRNA and a concomitant loss of FMRP, in FXTAS there is not a substantial alteration in FMRP levels – only a modest reduction in the high premutation repeat range (Fig. 1B). However, in premutation cases there is a dramatic increase in *FMR1* mRNA (Fig. 1B). There are three primary molecular mechanisms by which *FMR1* premutation excess mRNA is believed to lead to FXTAS neuropathology (Hagerman and Hagerman, 2016; Ma et al., 2019), including: 1) Sequestration of proteins and RNAs into inclusion bodies (Fig. 2D/E) that leads to impaired cell function due to loss of these RNA and protein species; 2) R-loop formation leading to DNA damage; and 3) RAN translation leading to the production of toxic FMRpolyG protein (Fig. 1B). In addition, elevated mRNA levels lead to elevated levels of Ca^{+2} in the neuron and subsequent mitochondrial dysfunction which worsens as FXTAS develops (Giulivi et al., 2016). These proposed mechanisms are not mutually exclusive, and there may remain other yet unidentified molecular mechanisms by which *FMR1* premutation leads to pathogenesis.

The first mechanism proposed for how FMR1 premutation leads to FXTAS is that *FMR1* premutation mRNA sequesters other RNA and protein species into intranuclear inclusion bodies, which in turns disrupts essential cellular processes dependent on these molecules. Inclusion bodies are the hallmark neuropathological indicator of FXTAS; they contain *FMR1* mRNA but lack FMRP (Ma et al., 2019; Iwahashi et al., 2006), and are exclusively located within the nucleus (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2004; Wenzel et al., 2010). Inclusions are almost always present as a single body (Greco et al., 2006; Greco et al., 2002; Wenzel et al., 2010; Hunsaker et al., 2011), with the exception of Purkinje cells that sometimes form twin inclusions (Ariza et al., 2016). FACS sorting in combination with inclusion autofluorescence has enabled improved isolation and purification of inclusion bodies, revealing some of their basic biochemistry (Ma et al., 2019). Inclusions are predominantly an aggregate of protein, composed of a heterogenous assortment of many proteins which are particularly enriched in RNA-binding, DNA-binding, and protein turnover regulating proteins (Ma et al., 2019). The inclusions also contain mRNA species, but to a much lower degree than protein, and inclusions do not contain DNA (Ma et al., 2019). Five proteins are particularly enriched: SUMO2, p62/SQSTM1, Myeloid Leukemia Factor 2 (MLF2), Ubiquitin, and Myelin Basic Protein (MBP) (Ma et al., 2019). The authors emphasize that the protein species found in the inclusion are indicative of inclusions forming in response to oxidative stress, and suggest that this may lead to an impairment in DNA damage response and an impairment in protein autophagy, both of which may lead to toxicity and neurodegeneration (Ma et al., 2019). However, while the specific mechanisms by which inclusion mediated protein sequestration leads to disease are an area of active investigation, these mechanisms remain poorly understood.

The second proposed molecular mechanism involves the formation of R-loops during *FMR1* premutation transcription leading to DNA damage. CGG expansion at the *FMR1* locus results in an increase in the number and size of R-loops formed (Loomis et al., 2014), during which time the non-template DNA strand is vulnerable to DNA damage (Roy and Lieber, 2009; Ginno et al., 2013). DNA damage should be corrected by the DNA damage response (DDR) molecular signaling pathway (Silva et al., 2014; Barzilai, 2010) that appears to be impaired in FXTAS (Kaplan et al., 2012), possibly through sequestration of DDR molecules by FXTAS inclusions (Ma et al., 2019; Iwahashi et al., 2006). Additionally, high levels of oxidative stress and mitochondrial dysfunction occurs in FXTAS (Kaplan et al., 2012; Alvarez-Mora et al., 2017), which could independently lead to further DNA damage, or possibly exacerbate DNA damage caused by R-loop formation.

The third proposed molecular mechanism in FXTAS is RAN (non-AUG) translation (reviewed extensively in Glineburg et al., 2018, Boivin et al., 2018). RAN translation is a common feature in triplet repeat expansion disorders, whereby regions of an mRNA containing the triplet repeat become prone to errors in translation and protein synthesis is initiated outside of the traditional AUG start codon (Sellier et al., 2017; Todd et al., 2013; Zu et al., 2011; Cleary et al., 2018; Cleary and Ranum, 2017) (Zu et al., 2011; Cleary and Ranum, 2017; Ayhan et al., 2018; Banez-Coronel et al., 2015). In FXTAS, the non-coding region of *FMR1* premutation mRNA is translated into multiple

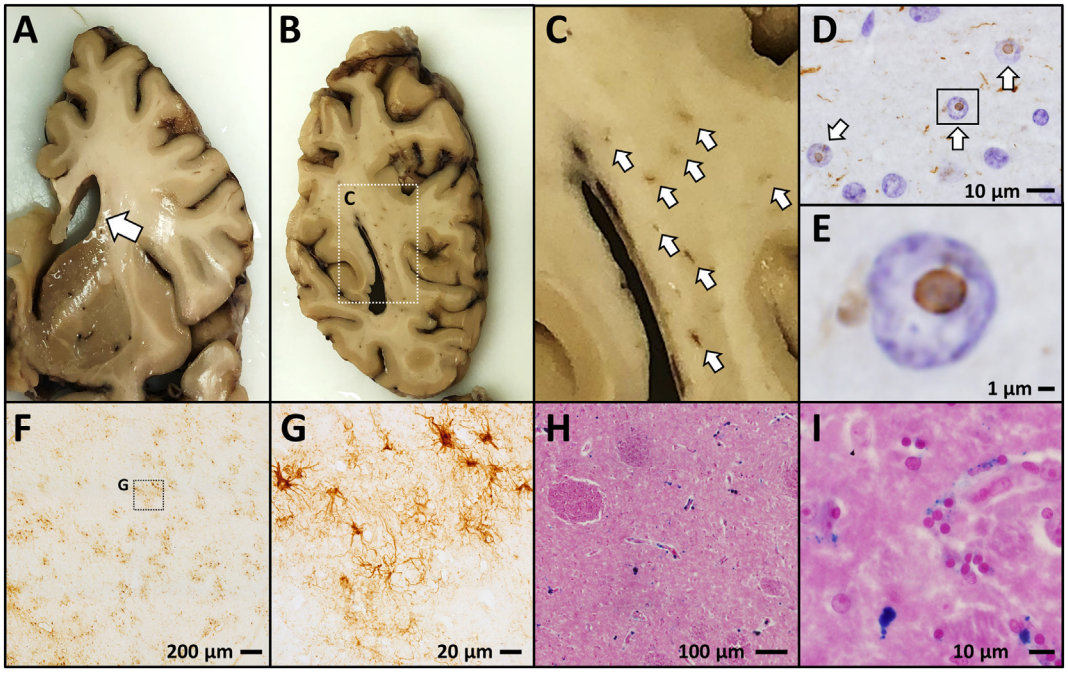

Fig. 2. FXTAS Neuropathology. Postmortem neuropathological analysis from human FXTAS cases show a characteristic neurodegenerative phenotype which includes ventricular enlargement (A); focal white matter lesions (B,C); ubiquitinated intranuclear inclusion bodies (D,E – ubiquitin IHC with hematoxylin nuclear counterstain); patches of astrogliosis (F,G - GFAP IHC); and excessive iron accumulation (H,I – ferric iron stained using Perl's method, Eosin counterstain).

RAN translation proteins, including the FMRpolyG protein being the most highly expressed and thus likely the largest contributor to pathology (Sellier et al., 2017; Todd et al., 2013). Sellier et al. (Sellier et al., 2017) reported that FMRpolyG protein is present in inclusion bodies that form in the cytosol, and that these FMRpolyG positive inclusions are subsequently transported into the nucleus where they disrupt the nuclear lamina protein LAP2β, leading to toxicity. They also reported that this toxicity only occurs when FMRpolyG proteins are produced from *FMR1* premutation mRNA in transgenic rodent models, and that increasing expression of LAP2β ameliorates FMRpolyG toxicity (Sellier et al., 2017). Numerous studies have identified the presence of intranuclear FMRpolyG+ and FMRpolyA+ inclusions in FXTAS postmortem human brain (Sellier et al., 2017; Krans et al., 2019; Sacino et al., 2019; Buijsen et al., 2014) and peripheral tissue (Buijsen et al., 2014; Buijsen et al., 2016), as well as in *FMR1* premutation rodent models (Sellier et al., 2017; Wenzel et al., 2019; Gohel et al., 2019). Multiple studies in FXTAS rodent, fly, and cell culture models have also demonstrated that expression of *FMR1* RAN translation products are necessary for inclusion formation and toxicity (Sellier et al., 2017; Todd et al., 2013; Hoem et al., 2019; Oh et al., 2015). However while it is clear that RAN translation does occur in humans with FXTAS, whether it is a central driver of pathogenesis remains unclear. In a recent study utilizing mass spectroscopy to analyze human FXTAS inclusion composition, Ma et al. (Ma et al., 2019) indeed detected RAN translation products in human FXTAS brain tissue, but also found using parallel reaction monitoring that they occurred in very low abundance and were not localized within FXTAS inclusion bodies. Future studies are needed to expand upon and further clarify the relative contribution of these three proposed mechanisms of *FMR1* premutation toxicity, in addition to identifying other possible molecular mechanisms that may also co-occur.

3.4. Neuropathology

The hallmark pathological change in FXTAS is the presence of intranuclear inclusion bodies (Fig. 2D/E) that are ubiquitin and *FMR1* mRNA positive (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2012b). FXTAS inclusion burden is positively correlated with CGG repeat length within the premutation range (Greco et al., 2006). In the CNS, FXTAS inclusions occur in astrocytes and neurons, although they tend to be larger and occur at a higher frequency in astrocytes in male cases (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2012b). There is a regional variability in the proportion of neurons and astrocytes bearing inclusions. At one extreme, there tend to be no inclusion + neurons in the pons, although inclusion+ astroctyes are abundant occurring in 9–30% of cells (Greco et al., 2006). Inclusion+ neurons and astrocytes are particularly prevalent in the hippocampus (up to 40% of both cell types) and to a lesser degree in the frontal and temporal cortex (≤10% of neurons and ≤ 20% of astrocytes) (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2012b). Neuronal and astrocytic inclusions also occur in the putamen, globus pallidus, substantia nigra, and amygdala (Greco et al., 2002; Louis et al., 2006). Inclusion bodies are also present in the periphery, including in the peripheral nervous system, enteric nervous system, endocrine glands, heart, and kidney (Hunsaker et al., 2011; Gokden et al., 2009).

Gross pathological assessment of the FXTAS brain indicates severe white matter disease, atrophy of both grey and white matter, and ventricular enlargement (Fig. 2A) (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2012b). Spongiosis and discoloration of cerebellar white matter is present in the vast majority of FXTAS cases (Fig. 2B/C) (Greco et al., 2006; Greco et al., 2002; Tassone et al., 2012b), including the MCP (Greco et al., 2006). Cerebral white matter disease is also common, but less consistently than in the cerebellum (Greco et al., 2006). Microscopic evaluation of white matter demonstrates a loss of myelin, as well as axonal degeneration (Greco et al., 2006). Grey matter atrophy of frontal cortex, cerebellum, and the pons have been qualitatively documented and consistently occur in FXTAS cases (Greco et al., 2006; Greco et al., 2002). While grey matter neuronal and possibly astrocytic cells loss is presumed, the relative contributions of cellular atrophy and cell loss to regional volumetric reductions have not been directly assessed. Grey matter neuronal loss has so far only been demonstrated to occur in Purkinje cells (Greco et al., 2006; Greco et al., 2002), and to date has not been systematically assessed in other brain regions or cell types. Female FXTAS cases that present clinically with

dementia symptomology also tend to show high rates of comorbid Alzheimer's neuropathology, including significant amyloid plaques and neurofibrillary tangles (Tassone et al., 2012b). Finally, postmortem FXTAS brains show high levels of iron accumulation in brain capillaries and parenchyma (Fig. 2H/I), as well as choroid plexus, which can occur as extracellular or intracellular deposits (Ariza et al., 2017; Ariza et al., 2015; Rogers et al., 2016). Striatal iron accumulation is prevalent and severe (Ariza et al., 2017), while cerebellar iron accumulation occurs in a smaller subset of patients (< 25%) and at lower levels (Rogers et al., 2016). The iron binding protein ceruloplasmin is also dramatically reduced in neurons and astrocytes, and to a lesser degree in oligodendrocytes (Ariza et al., 2017), suggesting that FXTAS associated dysregulation of iron metabolic pathways may underlie iron accumulation. In contrast, ceruloplasmin and transferrin expression is increased in microglia, as well as intracellular iron deposits, suggesting that microglia may be actively attempting to counteract iron accumulation (Ariza et al., 2017). Astrocytes show profound reactive gliosis and profound microglial activation occurs in a majority of FXTAS cases (Fig. 2F/G) (Martinez Cerdeno et al., 2018). Microglial senescence also occurs in FXTAS suggesting that disease-associated microglial impairment may further exacerbate FXTAS neuropathology (Martinez Cerdeno et al., 2018).

4. Conclusion

Many physicians confuse FXS and FXTAS so it is essential to remember that these are 2 very different disorders, one causing ID and autism and the other leading to neurodegeneration in otherwise normally developed individuals who are aging. Each disorder has differing levels of CGG repeats with deficient levels of FMRP in FXS and elevated levels of *FMR1* mRNA in FXTAS. However, these disorders are usually found in the same families and often multiple individuals with each of these disorders can be found. Therefore, when one individual is identified with a fragile X mutation the whole family through multiple generations are at risk for one or more of these mutations. Cascade testing for these mutations are necessary throughout the family tree either by the physician who identified the initial mutation or by referral to genetics so that the whole family can understand and be tested for these disorders.

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