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Neuropathological, Clinical, and Molecular Pathology in Female Fragile X Premutation Carriers with and without FXTAS

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Abstract

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an adult-onset neurodegenerative disorder associated with premutation alleles of the fragile X mental retardation 1 (*FMRI*) gene. Approximately 40% of older male premutation carriers, and a smaller proportion of females, are affected by FXTAS; due to the lower penetrance the characterization of the disorder in females is much less detailed. Core clinical features of FXTAS include intention tremor, cerebellar gait ataxia, and frequently parkinsonism, autonomic dysfunction, and cognitive deficits progressing to dementia in up to 50% of males.

Here, we report the clinical, molecular, and neuropathological findings of eight female premutation carriers. Significantly, four of these women had dementia; of the four, three had FXTAS plus dementia. Post mortem examination revealed the presence of intranuclear inclusions in all eight cases, which included one asymptomatic premutation carrier who died from cancer. Among the four subjects with dementia, three had sufficient number of cortical amyloid plaques and neurofibrillary tangles to make Alzheimer's disease a highly likely cause of dementia and a fourth case had dementia with cortical Lewy bodies. Dementia appears to be more common than originally reported in females with FXTAS. Although further studies are required, our observation suggests that in a portion of FXTAS cases and Alzheimer pathologies a synergistic effect on the progression of the disease may occur.

Keywords

FMRI premutation; FXTAS; Alzheimer Disease; RNA toxicity; Activation Ratio

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This work is dedicated to the memory of Matteo.

INTRODUCTION

The *FMR1* gene is highly polymorphic for CGG trinucleotide repeats in its 5' untranslated region. CGG expansions in the premutation range (55–200 repeats) give rise to the leading known single-gene cause of primary ovarian insufficiency (Oostra & Willemsen, 2009), and the late-onset neurodegenerative disorder, FXTAS (Hagerman *et al.*, 2001). FXTAS is characterized by intention tremor and gait ataxia, with both age-of-onset and severity being associated with the number of CGG repeats (Tassone *et al.*, 2007, Tassone & Berry-Kravis, 2010). Cerebral atrophy and white matter disease are prominent imaging findings in individuals with FXTAS (Adams *et al.*, 2007, Adams *et al.*, 2010). White matter MRI hyperintensities in the middle cerebellar peduncles (i.e., “MCP sign”) represent a major diagnostic criterion for FXTAS although observable in only 58% of males and 13% of female carriers (Adams *et al.*, 2007, Brunberg *et al.*, 2002). The pathological hallmark is the presence of intranuclear inclusions in neurons and astrocytes (Greco *et al.*, 2002), in the neuroendocrine system (Louis *et al.*, 2006), in the autonomic nervous system, and myocardial cells (Gokden *et al.*, 2008, Greco *et al.*, 2007, Hunsaker *et al.*, 2011).

The pathogenesis of premutation-associated disorders, particularly FXTAS, is thought to involve an RNA toxic gain-of-function mechanism, mediated by elevated levels of premutation length *FMR1* mRNA (Allen *et al.*, 2004, Kenneson *et al.*, 2001, Peprah *et al.*, 2010, Tassone *et al.*, 2000) which has been detected within the intranuclear inclusions present in the brain (Tassone *et al.*, 2004). Although the role of these inclusions in the pathogenesis of FXTAS remains unclear, it is likely that they reflect, a much broader process of cellular sequestration of one or more proteins whose functions are thereby compromised (Raske & Hagerman, 2009, Sellier *et al.*, 2010).

FXTAS occurs in approximately 40% of carrier males (Jacquemont *et al.*, 2004a) with an increased likelihood of penetrance with age (Jacquemont *et al.*, 2004b). A reduced FXTAS penetrance (between 8 and 17%) has been observed in female premutation carriers (Chonchaiya *et al.*, 2010a, Coffey *et al.*, 2008, Jacquemont *et al.*, 2004b, Rodriguez-Revena *et al.*, 2009). This may be primarily due to the protective effect of the normal allele on the second X chromosome (Berry-Kravis *et al.*, 2005, Jacquemont *et al.*, 2005). Indeed, female premutation carriers show less frequent clinical and neuropathological features than male premutation carriers, both with and without FXTAS, but in some cases they may have symptoms that are as severe as those of their male counterparts (Adams *et al.*, 2007). FXTAS females have a higher incidence of hypothyroidism, potentially autoimmune thyroid disease and fibromyalgia, than either age-matched controls or males with FXTAS (Coffey *et al.*, 2008, Leehey *et al.*, 2011, Rodriguez-Revena *et al.*, 2009). They also present hypertension, seizures, and peripheral neuropathy more often than controls (Chonchaiya *et al.*, 2010b, Coffey *et al.*, 2008, Hamlin *et al.*, 2011). Co-occurrence of multiple sclerosis (MS) has been described in women carriers (Greco *et al.*, 2008, Zhang *et al.*, 2009). We have studied eight premutation females and here we report their neuropathological findings, molecular data, and clinical problems.

MATERIALS AND METHODS

Molecular measures

DNA analysis—Genomic DNA was isolated from peripheral blood leucocytes (5 ml of whole blood) obtained prior to death, and from post-mortem sections of approximately 500mg of brain tissue using standard methods (Puregene Kit; Gentra Inc.) and under informed consent according to Institutional Review Board approved protocols. Southern blot analysis, PCR analysis and calculation of the repeat size for both methods were performed as described in Tassone *et al.* (2008). The Activation Ratio (AR), which expresses the

percent of cells carrying the normal allele on the active X chromosome, was measured using an Alpha Innotech FluorChem 8800 Image Detection System as previously described (Tassone *et al.*, 1999).

***FMR1* mRNA levels**—Total RNA was isolated from peripheral blood leukocytes using Tempus tubes (Applied Biosystems, Foster City, CA). Reverse transcriptase reactions and quantifications of *FMR1* mRNA were performed as described in Tassone *et al.* (2000).

Pathology

Formalin-fixed brain tissue for all cases was processed for paraffin sections, and histological and immunohistological staining in standard fashion as previous reported (Greco *et al.*, 2006, Greco *et al.*, 2002). Tissue blocks of frontal cortex and hippocampus were obtained from five age matched control female subjects (age range 60–76 years). Tissues were selected to be the same across all cases to facilitate comparisons among experimental cases. Control cases were obtained from pathology files at Department of Pathology at University of California, Davis according to UC Davis approved IRB protocol.

Evaluation of AD neuropathology

We evaluated the AD neuropathology, including the Braak and Braak stage and the CERAD plaque score, using the Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease, following the recommendation by the National Institute on Aging and Reagan Institute Working Group (NIA-Reagan criteria) (1997).

Quantitative analysis of intranuclear inclusion number

Percentages of intranuclear inclusions in neurons and astrocytes were determined for frontal cortex, hippocampus, and middle superior temporal gyrus using protocols previously described (Greco *et al.*, 2006, Greco *et al.*, 2002, Greco *et al.*, 2008). Tissue from blocks of frontal cortex and hippocampus were obtained for 8 female cases and middle superior temporal gyrus was obtained for 7 of these cases. Quantifications were compared to previously reported female control cases from Greco *et al.* (2006). These control cases matched the age range of the present female cases, and were free of gross neuropathological features or histological artifacts.

Brain tissues were blocked and sectioned in the same orientation to facilitate histological analysis. Tissues were paraffin embedded, sectioned at 10 μm , and stained with hematoxylin and eosin (H&E). Three sections separated by 50 μm were used for unbiased cell counting.

To estimate the number of intranuclear inclusions in females with the fragile X premutation both with and without FXTAS, regions of interest were outlined within the frontal cortex, superior medial temporal gyrus, and hippocampus, using the StereoInvestigator (v 8.0, MBI, Inc.; Williston, TN) software package and a Nikon E600 Eclipse microscope at 400x magnification. All parameters explicitly replicated those reported by Greco *et al.* (2006) quantifying inclusions presence in male FXTAS cases to facilitate comparison between the results of that study and the present report. To reduce bias in our samples, standard sections were taken from all control cases and premutation cases to allow direct comparisons among cases. Unbiased sampling techniques were used to collect and define a systematic, random sampling of the tissue to ensure an unbiased estimation of inclusion number, but no rigorous stereology was carried out in the present study and we are reporting the actual number of items counted, not estimated values provided by the software. Briefly, these techniques involved tracing the regions of interest at 40X magnification by an experimenter blinded to the identity of each case (i.e., slide blinded). Once the region of interest was traced, the software placed a regular grid of sampling frames across the region of interest using a

randomized starting point. The size of the counting frame and spacing of the grids were the same as reported in Greco *et al.* (2006). This way, there was a rigorous, systematic sampling of the area contained within the region of interest, but the experimenter did not have control over precisely which areas of the region of interest were sampled. Counting of cells containing intranuclear inclusions was performed at 400X. Cell identification within the regions of interest was based on standard morphological criteria as previously described (Greco *et al.*, 2006, Greco *et al.*, 2002). The numbers obtained from these cell counts were converted to ratios (# of cell type with inclusions/total # of cell type counted).

RESULTS

Clinical Histories

Key clinical features and molecular characteristics of the eight cases included in this study are presented in Table 1.

Case 1—Case 1 was an asymptomatic premutation carrier who had no neurological, behavioral, or cognitive symptoms by history. She had a 30 CGG repeat normal allele and 70 CGG repeat premutation allele with an activation ratio (AR) = 0.8. Her *FMRI* mRNA levels were 1.31 (+/- 0.11) times normal. At age 40, she experienced an episode of Bell's palsy. She had dry eyes resulting from cataract surgery in her early 70's. At age 73, she developed uterine cancer and had a hysterectomy, followed by radiation treatment and chemotherapy. She died of uterine cancer at age 76, after a downhill course involving weakness and cognitive decline terminally.

Case 2—Case 2 experienced her first symptoms at the age of 56 years, presenting with expressive and receptive aphasia. The aphasia was not acute and there was no indication of a stroke. The detailed work up ruled out vascular infectious or inflammatory etiology. She had a normal and a premutation allele of 30 and 80 CGG repeats and an AR = 0.3. Her *FMRI* mRNA levels were 6.06 (+/- 0.16) times normal. For this case, transcript levels were measured using frontal cortex as blood samples were not available. She subsequently developed hyperphagia, incontinence, and hallucinations. A positron emission tomography (PET) scan at age 61 showed hypoperfusion in left greater than right temporal lobes, and a brain MRI revealed mild cortical atrophy. Later, she experienced sudden falls and then swallowing difficulties. She died at 66 years of age after a progressive neurological and cognitive decline, and dementia.

Case 3—Case 3 had a history of mild FXTAS and was initially reported in Hagerman *et al.* (2004). She had a normal and a premutation allele of 29 and 87 CGG repeats, respectively, and an AR = 0.53. Her *FMRI* mRNA level was 2.31 (+/- 0.17) times normal. She was a social worker who had 2 children with FXS. She had a long history of anxiety and she developed depression at age 75, which was treated with fluoxetine. Mild ataxia began at age 79, necessitating use of a cane. She developed an intention tremor and extremity weakness at age 82 years. She required a pacemaker in her 80s for cardiac arrhythmias. She fell and fractured her left hip at age 84. She did not have any significant cognitive deficits except for impaired memory a few months prior to death. She died at age 85 from complications related to gastrointestinal surgery for ileus.

Case 4—Case 4 was originally reported in 2008 (Greco *et al.*, 2008), emphasizing the co-occurrence diagnosis of FXTAS and multiple sclerosis (MS). The *FMRI* alleles had 36 and 75 CGG repeats in size with an AR = 0.44. Her *FMRI* mRNA levels were 2.80 (+/- 0.12) times normal. She died of MS at age 52, having been found to be a carrier just 2 weeks before her death after her daughter was diagnosed as a carrier by her OB/GYN physician on

the basis of ovarian dysfunction. She developed neurological symptoms at age 32 years, with numbness in her arm and optic neuritis. By age 38 she had developed gait ataxia and loss of dexterity in the right hand. Her MRI demonstrated diffuse atrophy and multiple foci of increased T2 signal intensity in the periventricular and cerebellar white matter including the middle cerebellar peduncles (MCP sign). She was diagnosed with MS and treated with several regimens including methotrexate, but her symptoms gradually progressed. She developed an upper extremity intention tremor, progressive dysarthria, lower extremity numbness, and eventually spastic paraparesis and cognitive decline. She was unable to swallow and she became incontinent and bedridden before her death at age 52.

Case 5—Case 5 had normal and premutation alleles of 30 and 65 CGG repeats, respectively, and an $AR = 0.28$. No *FMRI* mRNA levels are available. Her medical history was unremarkable beside AD later in life. Her psychiatric history included anxiety and depression. At 73 years of age she was diagnosed with AD by her neurologist and started taking donepezil. At age 77 years, she was placed in an assisted living setting and a year later she moved to an AD nursing home unit after a fall resulting in a broken ankle. She was unsteady in her walking and had tremor before death. Her downhill course was rapid. She did not have an MRI. She died as a result of AD at age 80 as result of AD.

Case 6—Case 6 was a premutation carrier; the mother of two sons with FXS, who died of FXTAS at age 65. This case has been previously described by Yachnis and colleagues (Yachnis *et al.*, 2010). Her *FMRI* mRNA levels and number of CGG repeats are unknown as only fixed tissues were available and results of previous genetic testing were unknown. She had a history of fibromyalgia symptoms starting at age 40 years with pain in her extremities, she also had hypothyroidism, and menopause at age 45. At age 58 she developed handwriting difficulties because of an intention tremor. At age 59 years her tremor worsened and involved hands and arms bilaterally. Her left hand would spasm and would close with increased tone. She also developed bladder incontinence and restless leg syndrome between age 59 and 60. She subsequently became physically weaker, with cognitive decline beginning at age 60 years, leading to a diagnosis of dementia, which was said to be rapidly progressive after age 60 (Yachnis *et al.*, 2010). Her cognitive decline was characterized by getting lost, poor judgment and hygiene, eventually she lost the ability to converse. She required a walker at age 61 years due to ataxia, began using a wheelchair regularly at age 62, and by 63 years of age she was bedridden. Her MRI demonstrated white matter disease. She was hospitalized with aspiration pneumonia and died with respiratory failure.

Case 7—Case 7 died at age 84 years from FXTAS. She had two alleles, of 27 and 59 CGG repeats, and an $AR = 0.63$. Her *FMRI* mRNA levels were 1.83 (± 0.12) times normal. She had two daughters and one son with the premutation, and three grandchildren with FXS. She was well until age 67 when she developed hypertension. She developed breast cancer in her 70s and was treated with a lumpectomy and radiation. She subsequently developed a hand tremor at age 78 with deteriorating handwriting; she was diagnosed with Parkinson's disease and was treated with carbidopa/levodopa. Ataxia began at age 80 with frequent falling and cognitive decline that necessitated 24-hour care. By age 82 she did not recognize family members and spoke very little. She was diagnosed with dementia with Lewy Bodies (DLB); treatment with donepezil and memantine showed some clinical benefit. She gradually became weaker and was unable to walk independently after age 82 years; she then used a wheelchair consistently. On examination at age 83 she had an intention tremor, global weakness, and severe neuropathy with edema and absent vibration and pin prick sensation in her lower extremities. Her MRI demonstrated severe white matter disease in the pons,

caudate, putamen, and thalamus, in addition to global cerebral atrophy. There were ischemic changes in her basal ganglia with iron deposits in her cerebellar dentate nuclei.

Case 8—Case 8 was originally reported in 2004 as the fifth case of the original case series of women with FXTAS (Hagerman *et al.*, 2004). She had alleles of 30 CGG and 78 CGG repeats, and an AR = 0.21. Her *FMR1* mRNA levels were 2.6 (+/- 0.04) times normal. She developed episodes of lightheadedness, unsteady gait, and frequent falls at age 71 years. She also developed an intention tremor of the hand that improved first with propranolol and then more markedly with primidone. Subsequently she developed numbness in her lower extremities. At age 74 she had bradykinesia, dystonia of the neck muscles, and upper extremity rigidity in addition to an intention tremor and ataxia. Her symptoms gradually worsened and she died of respiratory failure at the age of 79.

Gross and microscopic findings

Case 1—Fresh brain weight was 1231 g. Gross examination of the brain showed no pathological changes. Comprehensive microscopic examination of the brain identified intranuclear inclusions in neurons and astrocytes (Figure 1D), and mild vascular hyalinosis of white matter. Neither neurofibrillary tangles (NFTs) changes or plaque formation was seen on the modified Bielschowsky stain (Braak and CERAD stage 0).

Case 2—Total brain weight (fixed) was 1140 g. Gross examination showed global atrophy, and diffuse cerebrovascular atherosclerosis. Microscopic examination of the brain showed intranuclear inclusions as established for the histological diagnosis of FXTAS (Figure 1A,B). Loss of cortical neurons, occasional senile plaques, granulovacuolar degeneration (GVD), and NFTs were identified in frontal and temporal cortices. The hippocampus showed prominent formation of NFTs, GVD, and senile plaques in CA1. Bielschowsky stain showed abundant plaques and NFTs conforming to Braak and Braak Stage V–VI and CERAD plaque assessment as “frequent”.

Case 3—Fresh brain weight was 980 g. Gross examination showed cerebral edema, and small, scattered, atheromatous plaques in the circle of Willis. Neurons and astrocytes demonstrated intranuclear inclusions throughout the central nervous system. There was patchy pallor of subcortical, deep, and periventricular white matter evident on both H&E and myelin stains, and patchy myelin pallor involved arcuate fibers in a patchy distribution. Bielschowsky stain showed few NFTs, plaques and GVD in hippocampus, and only rare plaques in cortical sections, consistent with Braak and Braak Stage I–II and a “sparse” CERAD plaque score.

Case 4—A detailed neuropathological description was presented previously in Greco *et al.* (2008). In brief, gross examination showed cortical atrophy and scattered, discrete plaques of demyelination throughout the brain. Histological evaluation identified characteristic features seen with multiple sclerosis, and the intranuclear inclusions in neurons and astrocytes described in FXTAS. Bielschowsky stain showed no NFTs or plaque formation.

Case 5—Brain weight was unavailable. Gross examination of the brain showed moderate frontal lobe atrophy, ventricular enlargement, and cerebrovascular atherosclerosis. Gray matter was thinned to 3 mm in all lobes. Intranuclear inclusions were present in neurons and astrocytes. Histological features seen corresponded to Braak and Braak Stage V–VI. The CERAD plaque count showed “frequent density” of neuritic and cored plaques.

Case 6—The brain weighed 1040 gm., and showed mild cerebrovascular atherosclerosis. Mild cortical atrophy was present, favoring frontal, insular, and temporal cortices, with

severe hippocampal atrophy. Neurons and astrocytes demonstrated intranuclear inclusions throughout the central nervous system (Figure 1C). Marked ventriculomegaly was seen, and some regions of white matter appeared almost translucent. Bielschowsky stain identified AD-like changes that corresponded to Braak and Braak Stage V–VI. According to CERAD criteria, plaques were “frequent” in density.

Case 7—Brain weight was unavailable. There was mild fronto-parietal cortical atrophy without ventriculomegaly, and mild spotty atherosclerosis in the Circle of Willis. Bielschowsky stain showed AD-like pathology that corresponded to Braak and Braak Stage I–II. Lewy bodies were seen in cortical areas and brainstem dopaminergic neurons.

Case 8—Brain weight was 930 gm., and showed moderate ventriculomegaly on cut section. The hippocampus was markedly atrophied. Histologic features as established for a post-mortem histological diagnosis of FXTAS were present. Bielschowsky stain identified AD-like pathological features corresponding to Braak and Braak Stage V–VI. “Moderate” numbers of plaques were present as per CERAD criteria. Severe arteriosclerosis was prominent in gray and white matter of frontal and temporal cortices.

Quantitative analysis of intranuclear inclusion number

The number of neurons and astrocytes with intranuclear inclusions (actual cell counts and percentages) are presented in Table 2 for frontal cortex, superior middle temporal gyrus, and hippocampus. No inclusions in neurons or astrocytes were observed for any of the similarly aged control subjects counted (n=6), and no inclusions were observed in the cytoplasm of neural cells in tissues from control or premutation cases. Intriguingly, the percentages of neurons and astrocytes with intranuclear inclusions were roughly equivalent in the female FXTAS cases, across all three areas evaluated (% \pm SEM) 9.03 \pm 1.62% neurons vs. 8.21 \pm 0.96% of astrocytes), which is in contrast to the findings previously reported in males (Greco *et al.*, 2006). Importantly, there were no differences for number of cells counted among the experimental cases and control tissues counted using the same techniques.

DISCUSSION

The two most significant findings of this study are the presence of intranuclear inclusions in all cases and a high number of them presenting with AD neuropathological changes. Female carriers of the fragile X premutation, both with and without a FXTAS clinical diagnosis, show similar numbers of intranuclear inclusions in neurons in the cortex and hippocampus as do men with FXTAS. The number of intranuclear inclusions in astrocytes, however, was greatly reduced in female carriers relative to the levels observed in male FXTAS cases (Greco *et al.*, 2006, Greco *et al.*, 2002). Additionally, we found evidence of intranuclear inclusions in all cases, even though one woman (Case 1) was asymptomatic. To our knowledge this is the first report of intranuclear inclusions in a fragile X premutation female carrier that showed no clinical symptoms associated with FXTAS. There also appeared to be little to no contribution of co-morbid disorders to the number of intranuclear inclusions in any of the female premutation and FXTAS cases. Two other striking findings were the high number of women carriers with dementia (Cases 2, 5, 6, and 7), much higher than expected given previous reports, and the high level of AD-type plaque and tangle pathologies (Cases 2, 5, 6, and 8), with or without corresponding clinical AD signs.

Importantly, we have demonstrated that although the presence of intranuclear inclusions is considered a primary diagnostic criterion for FXTAS (Hagerman *et al.*, 2004, Jacquemont *et al.*, 2003), the presence of intranuclear inclusions may be necessary but not sufficient to lead

to FXTAS symptoms. Two of the cases presented here did not have a FXTAS diagnosis at the time of death. Case 1 died of a uterine cancer for which she received radiation and chemotherapy. It is possible that the CNS trauma of the cancer treatment could have precipitated the formation of inclusions and FXTAS as was previously described in ODwyer *et al.* (2008) where chemotherapy lead to CNS white matter disease and clinical symptoms of FXTAS. However, the presence of the fragile X premutation appears to be sufficient for the presence of intranuclear inclusions, even when there are relatively normal levels of *FMR1* mRNA, as demonstrated by the findings in Case 1. It is possible that genetic and environmental factors, including the genetic background and a favorable AR, may have played a role in the absence of FXTAS symptoms in Case 1. A high AR (0.8) was in fact measured in Case 1 in both peripheral blood leukocytes and also in brain tissue. Case 5 had a history of anxiety and depression, which are common in carriers (Bourgeois *et al.*, 2009; Bourgeois *et al.*, 2011; Roberts *et al.*, 2009) and then presented with dementia with subsequent decline that included falling. Dementia has occasionally been a presenting sign of FXTAS and in Sevin *et al.* (2009) cognitive decline was more common in carriers compared to controls even in those without tremor and ataxia. Our cases demonstrate that the inclusions of FXTAS are more common than previously thought even in female carriers without classical symptoms of FXTAS.

Interestingly, cases 2, 5, 6, and 7 presented with dementia, which has only rarely been described in women with FXTAS. Only five women with FXTAS and dementia have been reported to date (Al-Hint *et al.*, 2007; Karmon & Gadoth, 2008; Rodriguez-Revenga *et al.*, 2009; Yachnis *et al.*, 2010). Thus, the present case series greatly enriches the existing literature with these additional three cases (cases 2, 6, and 7). In three of the women we described (Cases 2, 5, and 6), there is a high likelihood that dementia was due to AD lesions according to the Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease recommended by the National Institute on Aging and Reagan Institute Working Group (1997). The etiology of dementia in Case 7, who only had low levels of AD lesions, is not clear but the presence of Lewy bodies suggests a diagnosis of DLB (McKeith, 2006) or PD dementia. We have also seen the occurrence of FXTAS inclusions and Lewy bodies in one man with FXTAS (Greco *et al.*, 2002). It is, in fact, possible that individuals with FXTAS who developed cognitive changes may get diagnosed as having AD, PD, or DLB, since dementia in FXTAS has an interesting cortical-subcortical picture which may partially mimic other neurodegenerative dementias (Seritan *et al.*, 2008). Dementia associated with FXTAS has a characteristic profile different than other dementias, even though deficit severity may be of the order of the deficits encountered in AD (Bacalman *et al.*, 2006, Seritan *et al.*, 2008). Patients in more advanced stages of FXTAS (Gane *et al.*, 2010) develop both cortical (apraxia, memory recall, visuospatial skills impairment) and subcortical (memory retrieval, bradyphrenia, latency of speech, mood and personality changes) cognitive deficits, in conjunction with the movement disorders described (ataxia, tremor, bradykinesia, parkinsonian features). Although aphasia may be present, language is typically spared early in FXTAS (Grigsby *et al.*, 2007). Executive dysfunction is the most prominent deficit (Grigsby *et al.*, 2008, Grigsby *et al.*, 2007), it may occur prior to development of a full dementia and appears to mediate other cognitive deficits (Brega *et al.*, 2008). These women described with FXTAS and neurocognitive changes presented with a variety of clinical features, making a specific dementia diagnosis difficult. It is not clear whether all the cognitively impaired women described here had FXTAS dementia, since one was also diagnosed with AD and another, with DLB. However, co-occurrence of FXTAS with other neurological disorders may exacerbate symptom severity and contribute to a faster decline.

Although further studies using a larger series are required, we hypothesize that a previously unrecognized association may exist between the *FMR1* premutation and AD pathology.

There may be a synergy of having both disorders because both have mitochondrial dysfunction and both have oxidative stress and these problems could be amplified when they occur together (Ross Inta *et al.*, 2010; Napoli *et al.*, 2011; Ye *et al.*, 2011; Karbowski and Neutzner, 2012). However, this association could be independent of the development of FXTAS, as Case 5 developed AD without FXTAS. Because of the high frequency of female premutation carriers in the general population (1:110–259 females), the contribution of aberrant FMRP (*FMR1* protein) metabolism to the development of AD should be evaluated. FMRP controls amyloid precursor protein (APP) through inhibition (Darnell *et al.*, 2011) and FMRP may be somewhat lower in the brains of carriers (Handa *et al.*, 2005, Qin *et al.*, 2011) and if so APP levels may be higher in premutation carriers, as reported in Handa *et al.* (2005), possibly predisposing them to AD.

In summary, dementia is more common than had been previously observed in female premutation carriers, and the co-occurrence of AD-associated NFTs and plaques and FXTAS neuropathologic changes in this small series is a striking finding. Larger studies will be necessary to further substantiate this finding. The essential role of the premutation (even with normal *FMR1* mRNA levels) in the inclusion formation is a cardinal finding, which will also need to be further explored in subsequent studies.

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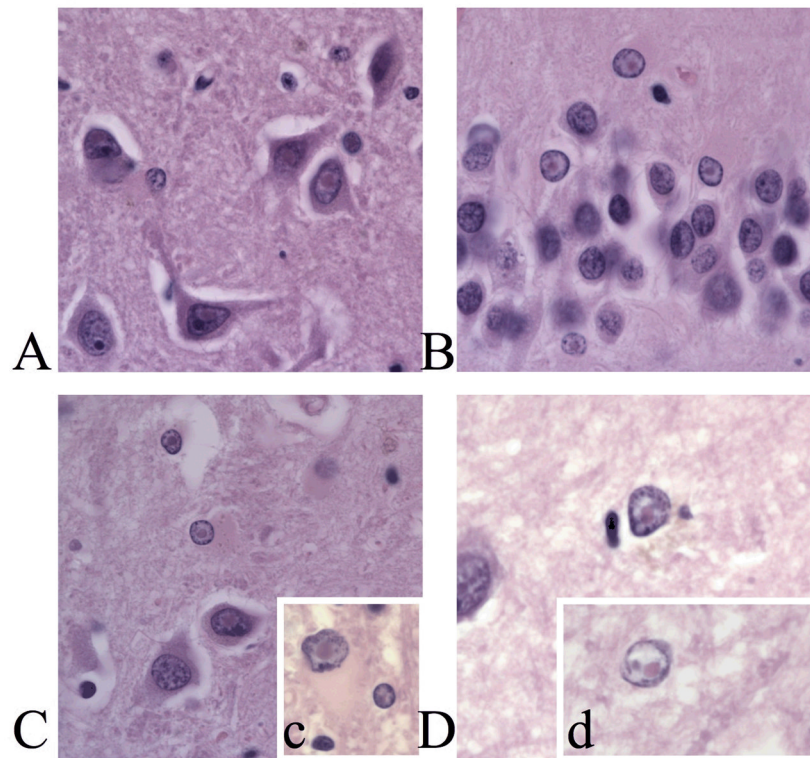


Figure 1.

A. Intranuclear inclusions in pyramidal cells in CA3b of Case 2. **B.** Intranuclear inclusions in granule cells of the medial blade of the dentate gyrus of Case 2. **C.** Intranuclear inclusions in pyramidal cells and neurons in the hilus of case 6. **c.** Inset, intranuclear inclusion in neuron and astrocyte in the white matter adjacent to layer VI of the frontal cortex of Case 6. **D.** Intranuclear inclusion in an astrocyte in layer II of the frontal cortex of Case 1. **d.** Inset, granule cell from layer IV of the frontal cortex in Case 1. All plates are at 1000X magnification. Scale bar = 50 μ m.

Table 1
Summary of the clinical, molecular and neuropathological features of the current cases

Case	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age of death	76	66	85	52	80	65	84	79
Symptoms	Bell's palsy, uterine cancer	Dementia	Slight intention tremor, cardiac arrhythmias	Cognitive decline; MS; neuropathy; tremor; ataxia	Dementia; tremor	Dementia; tremor, fibromyalgia, hyperthyroidism	DLB (2); intentional tremor, ataxia; neuropathy, hypertension; breast cancer	Intentional tremor; ataxia, neuropathy
FXTAS	No	Yes	Yes	Yes	No	Yes	Yes	Yes
CGG repeats	30,70	30, 80	29,87	36, 75	30, 63	Premutation(1)	27, 59	30, 78
Activation Ratio	0.80	0.30	0.53	0.44	0.28	-	0.63	0.21
FMRI mRNA levels	1.31 (0.11)	n/a	2.31 (0.17)	2.8 (0.12)	n/a	n/a	1.83 (0.12)	2.6 (0.04)
Intranuclear inclusions (FXTAS)	+	+	+	+	+	+	+	+
Braak & Braak staging (3)	0	Braak V-VI	Braak I-II	0	Braak V-VI	Braak V-VI	Braak I-II	Braak V-VI
CERAD plaque score (4)	0	frequent	sparse	0	frequent	frequent	sparse	moderate
Addl. significant histopathology	mild arteriolosclerosis	--	--	multiple sclerosis	--	--	Lewy bodies,	Severe arteriosclerosis

(1) Fixed tissues only; reported as premutation without allele sizes or AR (Yachnis et al., 2010)

(2) DLB - Dementia with Lewy body disease

(3) (Harding et al., 2000)

(4) (Mirra et al., 1991)

Table 2

Number of intranuclear inclusions (counts and percent) in neurons and astrocytes.

Subject		Gray Matter		White Matter	
		% Neurons	% Astrocytes	% Neurons	% Astrocytes
Case 1	24/265	9.06	25/680	3.68	30/346
Case 2	16/190	8.40	2/117	1.80	21/615
Case 3	2/231	0.80	5/200	2.50	19/333
Case 4	0/370	0.00	14/504	2.80	12/662
Case 5	6/251	0.02	10/403	0.02	9/501
Case 6	62/456	13.60	240/1729	13.90	38/333
Case 7	18/221	8.14	21/746	2.82	23/552
Case 8	17/358	4.75	45/701	6.42	23/299
<i>Mean</i>	--	5.6	--	4.24	--
<i>Controls</i>	0/361	0	0/251	0	0/303

Subject		Pyramidal Cell Layer (CA1-CA3)		Granule Cell Layer (DG)		Hilus		Superior Medial Temporal Gyrus	
		% Neurons	% Astrocytes	% Neurons	% Astrocytes	% Neurons	% Astrocytes	% Neurons	% Astrocytes
Case 1	11/361	3.05	38/496	7.66	13/90	14.44	23/537	4.66	27/384
Case 2	1/118	0.90	9/75	12.00	1/12	8.30	15/109	13.80	--
Case 3	7/148	4.70	4/127	3.10	6/372	1.70	17/117	14.50	25/347
Case 4	0/538	0.00	0/239	0.00	0/1094	0.00	0/462	0.00	26/298
Case 5	8/289	2.77	17/364	4.67	28/806	3.47	6/332	1.81	16/316
Case 6	79/332	24.00	157/698	22.00	100/899	11.00	205/831	25.00	46/234
Case 7	36/486	7.41	9/123	7.32	27/486	5.56	14/209	6.70	20/297
Case 8	26/263	9.89	72/514	14.01	68/777	8.75	101/901	11.21	52/236
<i>Mean</i>	--	6.59	--	8.84	--	11.56	--	9.71	--
<i>Controls</i>	0/109	0.00	0/165	0.00	0/483	0.00	0/280	0.00	0/599