Published in final edited form as:
Acta Neuropathol. 2011 October ; 122(4): 467–479. doi:10.1007/s00401-011-0860-9.

Widespread non-central nervous system organ pathology in fragile X premutation carriers with fragile X-associated tremor/ataxia syndrome and CGG knock-in mice

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Abstract

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an adult-onset neurodegenerative disorder generally presenting with intention tremor and gait ataxia, but with a growing list of co-morbid medical conditions including hypothyroidism, hypertension, peripheral neuropathy, and cognitive decline. The pathological hallmark of FXTAS is the presence of intranuclear inclusions in both neurons and astroglia. However, it is unknown to what extent such inclusions are present outside the central nervous system (CNS). To address this issue, we surveyed non-CNS organs in ten human cases with FXTAS and in a CGG repeat knock-in (CGG KI) mouse model known to possess neuronal and astroglial inclusions. We find inclusions in multiple tissues from FXTAS cases and CGG KI mice, including pancreas, thyroid, adrenal gland, gastrointestinal, pituitary gland, pineal gland, heart, and mitral valve, as well as throughout the associated autonomic ganglia. Inclusions were observed in the testes, epididymis, and kidney of FXTAS cases, but were not observed in mice. These observations demonstrate extensive involvement of the peripheral nervous system and systemic organs. The finding of intranuclear inclusions in non-CNS somaticorgan systems, throughout the PNS, and in the enteric nervous system of both FXTAS cases as well as CGG KI mice suggests that these tissues may serve as potential sites to evaluate early intervention strategies or be used as diagnostic factors.

Keywords

FXTAS; Intranuclear inclusions; Fragile X premutation; CGG KI mouse

Introduction

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder that is characterized by cerebellar gait ataxia and intention tremor. FXTAS affects carriers of a fragile X mental retardation 1 (FMR1) gene containing a CGG trinucleotide repeat expansion in the 5′ untranslated region (UTR) within the premutation range (PM; 55–200 CGG repeats). Premutation length CGG trinucleotide repeat expansions are present in as many as 1:251 males and 1:116 females in the population [4, 24, 56]. Both male and female PM carriers older than 50 years of age may develop FXTAS, although the incidence of FXTAS in female PM carriers is less than half of males (~40% of male PM carriers vs. 8–16% of female PM carriers develop FXTAS [9, 24, 45, 64]), primarily thought due to a protective effect of the non-expanded FMR1 gene on the second X chromosome. Premutation carriers produce elevated levels (2–10 times normal measured in lymphocytes) of PM FMR1 messenger RNA (mRNA) and normal to moderately reduced levels of FMR1 protein (FMRP) in leukocytes [4, 57, 58, 70], fibroblasts [29], and brain tissue [71]. The current hypothesis underlying the pathophysiology of FXTAS focuses on a toxic mRNA gain-of-function mechanism [28].

The onset of FXTAS symptomatology begins at a mean age of 60 years, and penetrance is age-dependent [45, 52]. The core features of FXTAS include progressive intention tremor and cerebellar gait ataxia [8, 10, 11, 35, 44]. Radiologic evaluations using magnetic resonance imaging (MRI) have identified generalized atrophy of the cerebrum, brainstem, and cerebellum in FXTAS [1, 2, 25], and a majority of male FXTAS patients show distinctive, bilateral, white matter signal hyperintensities in the middle cerebellar peduncles on T2-weighted or FLAIR MRI scans [21, 53]. These radiologic features have been histologically confirmed in multiple post mortem cases [31–34, 71].

Associated clinical features of FXTAS include cognitive decline, Parkinsonism, peripheral neuropathy, and autonomic dysfunction [23, 35, 36, 38, 46, 54, 55, 61, 67, 77]. The neuropathological hallmark of FXTAS is the presence of eosinophilic, ubiquitin-positive intranuclear inclusions in both neurons and astroglia throughout brain upon post mortem histological analysis [30–34, 77]. These inclusions appear as eosinophilic, hyaline, refractile, 2–5 μm diameter, round to ovoid bodies that show positive reactivity with antibodies against over 20 different proteins including ubiquitin, αB-crystallin, lamin A/C, hnRNP A2, myelin basic protein, DNA repair-ubiquitin-associated HR23B, and Sam68, among others [6, 27, 43, 59, 65]. The inclusions are PAS, silver, amyloid, and α-synuclein negative [31, 32]. Additionally, the FMR1 mRNA, but not FMRP, has been found contained within intranuclear inclusions [43, 72]. Additional neuropathological features present in FXTAS include reduced Purkinje cell number, axonal torpedoes, and prominent cortical and subcortical white matter pathology [31, 32].

The CGG knock-in (CGG KI) mouse model of the PM has proven to be an invaluable tool for the study of the pathophysiology of FXTAS, including neuropathological events that occur with the onset and progression of the disorder (cf., [7]). The CGG KI mouse model was developed by homologous recombination wherein the endogenous mouse $(CGG)_8$ trinucleotide repeat within the mouse Fmr1 gene was replaced by a $(CGG)_{98}$ repeat of human origin. As such, expression of the expanded $(CGG)_{98}$ repeat is on the endogenous mouse Fmr1 promoter [76]. The CGG KI mouse recapitulates many neuropathological features present in FXTAS, including intranuclear inclusions in neurons and astroglia, elevated Fmr1 mRNA levels, and reduced Fmrp levels [17–20, 76] (cf., [26, 59]) for a different knock-in model of the PM demonstrating similar molecular features. In addition, a correlation has been demonstrated between the presence of intranuclear inclusions in brain and phenotypes in CGG KI mice that model the clinical features of FXTAS [18, 40–42,76]. Furthermore, CGG KI mice show an increase in neurocognitive dysfunction both with increasing age and CGG repeat length across a growing battery of behavioral tests [40–42, 73].

It has become apparent that the spectrum of clinical involvement in PM carriers with FXTAS extends beyond symptoms and signs that correspond to pathology in the CNS [10, 11, 14–16, 24, 30, 33, 67]. The increasingly broad clinical spectrum of FXTAS symptomatology seems to encompass a number of medical co-morbidities that include thyroid disease [24, 64], fibromyalgia [24], gastrointestinal symptoms [12, 37] hypertension [24], migraine [3, 12], impotence [33], autoimmune disease [24,51], peripheral neuropathy [36, 67], seizure disorders [5], and cardiac arrhythmia [35, 44]. Intriguingly, evidence for this broadened spectrum of immune mediated disorders [24, 51] arise primarily in women both with and without FXTAS, but many of the medical co-morbidities are more commonly observed in individuals with FXTAS comparedto PM carriers without FXTAS [24, 30, 33, 67]. Furthermore, intranuclear inclusions have been identified in non-CNS tissues, but to date have only been evaluated in a limited number of cases [30, 33, 54]. Here we report the presence of inclusions in autonomic ganglia throughout the peripheral autonomic nervous system, as well as in somatic tissues themselves from ten PM carriers with FXTAS (9 male, 1 female), and compare these findings to homologous pathological features present in the CGG KI mouse model of the fragile X PM.

Materials and methods

FXTAS case autopsies

Clinical history reports for all FXTAS cases included in this report are available in Table 1, along with molecular correlates of the PM. Written informed consent was received for all cases and all experimental protocols conformed to IRB approved protocols. Tissues (pancreas, kidney, brain, thyroid gland, heart, testes, adrenal gland, gastrointestinal, and esophagus) were removed from a subset (cf., Table 1) at autopsy and immersion fixed in 10% formalin, followed by paraffin embedding of representative samples. The brain was also removed from each autopsy case, as was the spinal cord on select cases. In standard fashion, histological sections (5 μm) were stained by hematoxylin and eosin (H&E) for routine histological examination, and immunoperoxidase labeling was performed using rabbit antibodies targeted against ubiquitin (Dako, ZO458, Carpinteria, CA, USA) and counterstained with hematoxylin.

Mitral valve biopsy tissue

A1 x 1 mm biopsy was taken from the mitral valve of Case 8 during surgery, placed in phosphate-buffered saline for 2 h, postfixed in freshly made 4% phosphate-buffered paraformaldehyde for 2 h, and cryoprotected in 30% sucrose. Microtome sections (30 μm) were taken and stained using iron hematoxylin and eosin for histological analysis as well as a modified Van Gieson’s stain (Van Gieson’s stain with the addition of Eosin Y) to further characterize specific cell and tissue types. Immunostains were performed using rabbit antibodies targeted against ubiquitin (DAKO, ZO458; 1:2,000) and counterstained with hematoxylin.

CGG KI mice

The generation of the expanded CGG mice used in this study has been described previously [76]. For the current study, male mice with repeat sizes between 100 and 150 CGG repeats were used (all experiments), as well as 1 female mouse with 8 and 150 CGG repeats (mitral valve to compare with biopsy material from Case 8). All experiments were carried out in accordance with approved animal use protocols (Erasmus MC; University of California, Davis). CGG repeat lengths were determined as described previously [18, 42]. All mice used in this study were between 48 and 90 weeks of age at time of killing.

Tissues (pancreas, pituitary, heart, kidney, brain, thyroid gland, testis, adrenal gland, and intestine) were dissected immediately following cervical dislocation and fixed overnight in 4% paraformaldehyde at 4°C. Subsequently, tissues were embedded in paraffin according to standard protocols. Paraffin sections (7 μm) were cut and mounted on gelatin-coated slides. Immunostains were performed with rabbit antibodies against ubiquitin (Dako, ZO458; 1:500) followed by indirect immunoperoxidase labeling with hematoxylin counterstain. For co-localization studies, antibodies targeted against somatostatin and glucagon were combined with ubiquitin labeling using immunofluorescence techniques and counterstained with DAPI to identify cell nuclei.

For experiments evaluating CGG KI heart, mitral valve, and pineal gland, the heart was dissected from a CGG KI mouse immediately following cervical dislocation and immersion fixed in 4% paraformaldehyde for 2 h at 4°C, then transferred overnight into 30% sucrose at 4°C as a cryoprotectant. Concurrently, the brain was trans-aortically perfused with 12 mL of potassium shifted Ringer’s solution, followed by 60 mL of 4% paraformaldehyde over 20 min via gravity feed, gently removed from the skull so as not to damage the pineal gland, and transferred into 4% paraformaldehyde at 4°C for 1 h postfixation, followed by 10 and 30% sucrose solutions. The heart and brain, including the pineal gland were sagittally sectioned at 30 μm on a freezing stage microtome and a set of every fifth section was mounted on gelatin-coated slides and stained for H&E. Another section set of heart tissue was stained using a modification of Van Gieson’s stain similar to that done for the human aorta tissue. Indirect immunoperoxidase staining was performed on a third set of free floating sections using polyclonal antibodies targeted to ubiquitin (Dako, ZO458; 1:2,000), after which tissues were mounted on gelatin-coated slides, counterstained with hematoxylin, dehydrated, and coverslipped with Permount resinous mounting media.

Results

Human FXTAS cases

A summary of the organs in which intranuclear inclusions were identified across all cases of FXTAS is presented in Table 2. A brief summary is presented below focusing on theintranuclear inclusions identified in each organ, as well as rough percentages of cell nuclei in which inclusions were present. Novel pathological findings are illustrated in Fig. 1.

Heart—Intranuclear inclusions were identified in cardiomyocytes in the heart and surrounding autonomic ganglia in Cases 4, 6, and 7 (3–5% of cardiomyocytes), as well as in smooth muscle cells of the mitral valve (~1–2% of cells) and autonomic ganglia in the tunica externa from Case 8 (~5% of cells; Fig. 1a).

Pineal—Intranuclear inclusions were identified in pinealocytes and ganglion cells in the pineal gland of Case 1 (1–2% of cells; Fig. 1b).

Colon—Intranuclear inclusions were identified in smooth muscle cells, as well as in neurons of the submucosal and myenteric plexi of the rectum (1–2% of cells; Fig. 1c), sigmoid colon, and appendix of Cases 2, 3, and 4.

Kidney—Intranuclear inclusions were identified in mesangial cells and epithelial cells of the distal tubules of the kidney in Cases 1 and 6 (3–5% of cells; Fig. 1d).

Thyroid—Intranuclear inclusions were identified in the follicular and parafollicular cells in the thyroid glands of Cases 1 and 6 (~1% of cells; Fig. 1e).

Pancreas—Intranuclear inclusions were identified in Islets of Langerhans cells in the pancreas of Cases 4 and 6. The precise cell type harboring inclusions was not determined in human cases due to autolytic change (5–10% of cells; Fig. 1f).

Adrenal gland—Intranuclear inclusions were identified in the medullary cells of the adrenal gland, as well as in periadrenal ganglia of Cases 1, 3, 4, and 5 (1–2% of cells).

Esophagus—Intranuclear inclusions were identified in neurons of the myenteric plexus of the esophagus of Case 7 (1–2% of cells).

Testes—Intranuclear inclusions were identified in Leydig cells, smooth muscle cells, and nurse cells (1–2% of cells) in the testes of Cases 1, 2, 3, 5, and 9, supporting previous reports of inclusion presence in the testes [33].

Epididymis—Intranuclear inclusions were identified in the epithelial cells of the distal tubule of Case 5 (1–2% of cells).

Pituitary—Intranuclear inclusions were identified in basophiles, chromophobes, and acidophiles of the anterior pituitary, and pituicytes of the posterior pituitary of Cases 3 and 10 (1–2% of cells).

CGG KI Mouse

A summary of organ sites of intranuclear inclusions were identified in CGG KI mice compared with the findings in FXTAS is presented in Table 2. A brief summary is provided below organized in a similar manner to the human results (Fig. 2).

Heart—A considerable number of cardiac muscle cells contained ubiquitin-positive intranuclear inclusions in 48- to 72-week-old CGG KI mice (~2–3% of cells; Fig. 2a) with some being so large as to occupy nearly the entire nucleus of the cell. Intranuclear inclusions were not conclusively identified in the smooth muscle of the mitral valve, but were numerous in the autonomic ganglia in the tunica externa (~5% of cells).

Pineal gland—Intranuclear inclusions were present in pinealocytes, astrocytes, and ganglion cells in the pineal gland (~2–3% of cells; Fig. 2b).

Colon—Intranuclear inclusions were identified in myenteric ganglia in the colon (10% of cells; Fig. 2c).

Adrenal gland—Ubiquitin-positive intranuclear inclusions were present in chromaffin cells of the adrenal gland (5–10% of cells; Fig. 2d).

Thyroid—In thyroid tissue from CGG KI mice, the gland structure was normal in H&E sections. Further examination revealed the presence of ubiquitin-positive intranuclear inclusions in a significant number of parafollicular cells that secrete calcitonin (3–5% of cells; Fig. 2e).

Pancreas—In pancreatic tissue from CGG KI mice, the general histological features on H&E staining were similar to those of normal age-matched controls. However, in sections that were immunostained for ubiquitin we could detect ubiquitin-positive intranuclear inclusions in specific cells of islets of Langerhans (3–5% of cells). To determine which cell types contained intranuclear inclusions (Fig. 2f), costaining for somatostatin and ubiquitin as well as glucagon and ubiquitin were carried out (Fig. 2g). Intranuclear inclusions were found in the somatostatin producing D cells of the islets of Langerhans as well as glucagon producing A cells (Fig. 2h).

Pituitary—Intranuclear inclusions were identified in the anterior and intermediate pituitary in CGG KI mice with more in the pars intermedia (~58% of cells) than the pars anterior (18% of cells). Few inclusions were detected in the pars posterior (<1% of cells) [48]. The precise cell types harboring inclusions were not determined.

Testes—No inclusions were detected in the testes of CGG KI mice.

Kidney—No inclusions were detected in the kidney of CGG KI mice.

Discussion

The present results demonstrate pathological features in broad distribution within the peripheral autonomic nervous system as well as neuroendocrine and somatic organs in PM carriers with FXTAS. These findings are consistent with the expanding range of co-morbid medical features reported in FXTAS that include neuroendocrine dysfunction [24, 68], impotence [33], gastrointestinal symptoms [50], cardiac arrhythmias [35, 44], peripheral neuropathy [36, 66], and bladder dysfunction [12, 24, 33, 37, 44, 54,64, 67]. Type II diabetes has not been formally established as an associated clinical feature of FXTAS; however, there is ample anecdotal evidence from case histories that a substantial number of patients develop type II diabetes during their lifetime (cf., Cases 2, 5, 6, 9). Because the incidence of hypothyroidism and other thyroid disorders (cf., Case 8), hypertension (cf., Cases 4, 5, 6, 7, 8, 9, 10), peripheral neuropathy (cf., Cases 1, 4, 5, 6, 7, 8, 9, 10), and fibromyalgia have been reported to be higher in PM carriers with FXTAS as compared to age-matched non-PM carriers, these diseases may well be part of the syndrome of FXTAS, or at least associated medical features [24, 64]. In addition, cardiac arrhythmias (cf., Cases 5, 6) and gastrointestinal problems including constipation are commonly encountered in carriers with FXTAS [12, 37] (cf., Case 9). Previously, Louis et al. [54] reported inclusions in pituitary tissue (hypophysis) from one patient with FXTAS, and Gokden et al. [30] reported inclusions in a number of peripheral tissues including autonomic ganglia of the mesenteric plexus, pericardial tissue, adrenal tissue and paraspinal ganglia. The present report has expanded upon these findings in a larger group of FXTAS cases. Greco et al. [13, 14, 33] have reported inclusions in the Leydig cells of the testes of men who died of FXTAS and proposed that these inclusions may likely be related to the lowered testosterone levels and impotence seen in these men since the Leydig cells produce testosterone. Impotence is common in PM males (cf., Cases 7, 9), often becoming apparent even before the development of intention tremor or cerebellar gait ataxia related to FXTAS.

Psychiatric problems, particularly anxiety and depression, are CNS-associated disorders that are increased in PM carriers with and without FXTAS compared to controls [14, 16] (cf., Cases 2, 5, 6, 8, 10). These disorders may well be elicited by a combination of stress and environmental factors [13, 14], particularly as these factors affect the hypothalamic–pituitary–adrenal (HPA) axis. In addition to the CNS component, widespread peripheral pathology have been observed in the present study, namely the presence of intranuclear inclusions identified throughout the HPA axis, pineal gland, cardiac conduction system, peripheral nerves and autonomic ganglia, thyroid, digestive system, testes, and pancreas. It is quite likely that the medical co-morbidities in systemic organs and the autonomic nervous system share a common pathogenesis with that observed in the CNS of patients with FXTAS (i.e., mRNA toxicity), and thus may be considered non-CNS-associated features of FXTAS [15, 16, 24, 39, 62–64]. The presence ofintranuclear inclusions in somatic organs as observed in the present study are not the cause of these conditions comorbid with FXTAS, but rather signal organ systems affected by the PM FMR1 mRNA. Specifically, inclusions capture and sequester important proteins necessary for normal function of these cells, including splice factors [66] that may negatively affect organ function.

The broad distribution of intranuclear inclusion formation reported herein further expands the cell types and body systems that may be affected by RNA toxicity and signals new areas for investigation of disease pathology in PM carriers both with and without FXTAS symptomatology. For instance, the prevalence of type II diabetes and hypoglycemic episodes should be investigated in those with the PM and FXTAS compared to the general population, as it appears likely that insulin production may be reduced in FXTAS (cf., Cases 2, 5, 6, 9). The presence of intranuclear inclusions in the Islets of Langerhans may signal disease processes in the pancreas, namely potential RNA toxicity—a potential that warrants further investigation. Such investigations, both in the human and in the CGG KI mouse model, would underscore the value of studying the basic disease mechanisms in non-CNS tissues that are readily available through surgical and biopsy tissues.

The CGG KI mouse was also evaluated for the presence of inclusions in the same organ systems as the cases with FXTAS, and showed a strikingly similar pattern of inclusion formation in somatic organs and the autonomic nervous system. These results verify that CGG KI mouse models the somatic pathologic anatomical features present in FXTAS, in addition to modeling the neuropathological features of FXTAS [19, 20, 40, 75, 76]. This parallel non-CNS pathology underscores the utility of the mouse model for studying the pathogenesis and progression of non-CNS disorders associated with FXTAS.

Our understanding of the molecular mechanisms of RNA toxicity is evolving, and includes dysregulation of a number of proteins such as lamin A/C and heat shock proteins including αß crystallin [22, 28, 60]; sequestration of Sam68 and the dysregulation of the protein products of mRNAs, whose splicing is modulated by Sam68 [65]. Most recently, Ross-Inta et al. [65] demonstrated mitochondrial abnormalities in fibroblasts and brain tissue in PM carriers both with and without FXTAS. It is not clear what cellular processes underlie inclusion formation; and it is not known whether the inclusions are themselves toxic or simply reflect underlying cellular dysfunction. Nevertheless, intranuclear inclusions clearly provide a cellular marker or signal for the disease process underlying FXTAS and associated non-CNS disease. Understanding the breadth of pathological involvement in the peripheral tissues in the PM and FXTAS expands our understanding of the spectrum of medical disease associated with FXTAS.

Recent publications [30, 54] along with our findings place the neurodegenerative condition associated with FXTAS among the neurodegenerative disorders with involvement of the peripheral nervous system [47, 74], and in some cases, the neuroendocrine system and/or visceral organs. Among these disorders are the Lewy body diseases, including Parkinson’s disease and diffuse Lewy body disease [48], multiple system atrophy [56, 69], and neuronal intranuclear inclusion disorder [49]. These disorders are marked by the presence of non-CNS inclusions similar in appearance to those found in the brain and spinal cord. Other polyglutamine disorders showing peripheral inclusions are Machado-Joseph disease/ spinocerebellar ataxia type 3 [78] and spinal and bulbar muscular atrophy [74]. The similarity between the presence of inclusions in peripheral and somatic tissues in FXTAS and numerous other inclusion-bearing disorders suggests the inclusions may not cause the medical co-morbidities reported in FXTAS, but rather signal tissues affected by the mRNA toxicity associated with the PM. Furthermore, as the mutation underlying the PM occurs in the 5′ untranslated region of the FMR1 gene, the FMR1 protein (FMRP) is structurally normal, despite the expanded CGG repeats present in the FMR1 mRNA.

Further research is needed to understand the complexity of co-morbid medical problems associated with the PM and how these may be related to RNA toxicity. The finding of intranuclear inclusions in non-CNS somatic organ systems, throughout the PNS, and in the enteric nervous system of both FXTAS cases as well as CGG KI mice suggests that these tissues may serve as potential sites to evaluate early intervention strategies or be used as diagnostic factors. Success in this effort may assist clinicians to confirm a FXTAS diagnosis prior to designing interventions for individuals with the PM, PM-associated diseases, or early FXTAS.

Acknowledgments

This work was supported by National Institute of Health (NIH) grants HD036071, HD056031, NS044299, AG024488, HD02274, MH77554, MH078041, RL1 AG032115, RL1NS062411, and RL1 AG032119; the National

Fragile X Foundation; the Circle of Service Foundation; and the M.I.N.D. Institute. This work was also made possible by a grant (UL1 DE019583) from the National Institute of Dental and Craniofacial Research (NIDCR) in support of the NeuroTherapeutics Research Institute (NTRI) consortium; and by a grant (UL1 RR024146) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research.

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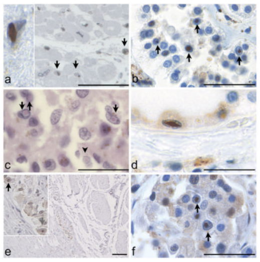

Fig. 1.

a Intranuclear inclusions in cardiomyocytes in Case 7 (x400; Immunoperoxidase (IP) stain). *Insert* Extremely large, oval-shaped inclusion in a cardiomyocyte from Case 6 (x1,000). **b** Intranuclear inclusions in pinealocytes and astrocytes in pineal gland of Case 1 (x1,000; H&E stain). **c** Autonomic ganglion of the myenteric/Auerbach's plexus is seen between longitudinal and circular muscular layers of the rectosigmoid colon (x40; IP stain) in Case 4. *Insert* Higher magnification identifies intranuclear inclusions in ganglion cells (x400). **d** Intranuclear inclusions in cells of the distal tubule of the kidney in Case 6 (x1,000; IP stain). **e** Intranuclear inclusions in thyroid of Case 6 (x1,000; IP stain). f Intranuclear inclusion in pancreas of Case 6 (x1,000; IP stain). All immunoperoxidase stains counterstained with hematoxylin. All *scale bars* represent 100 μm

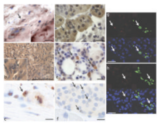

Fig. 2.

a Intranuclear inclusions in cardiomyocytes of CGG KI mice (x1,000; IP stain). **b** Intranuclear inclusions in pinealocytes of CGG KI mouse pineal gland (x1,000; IP stain). **c** Intranuclear inclusions in ganglion cells of the myenteric plexus of the colon in CGG KI mice (x1,000; IP stain). **d** Intranuclear inclusions in adrenal gland of CGG KI mice (x1,000; IP stain). **e** Intranuclear inclusions in thyroid of CGG KI mice (1,000x; IP stain). **f** Intranuclear inclusions in pancreas of CGG KI mice (x1,000; IP stain). **g** Immunofluorescence of intranuclear inclusions (*red*) and somatostatin (*green*) in pancreas of CGG KI Mice (nuclei counterstained with DAPI (*blue*)) (x1,000; IP stain). **h** Immunofluorescence of intranuclear inclusions (*red*) and glucagon (*green*; IP stain) in pancreas of CGG KI mice (nuclei counterstained with DAPI (*blue*)) (x1,000). All *scale bars* represent 50 μm

Table 1

Clinical history and molecular characterizations of all FXTAS cases and tissues samples evaluated from each in the present study

FXTAS Case	CGG	FMR1 mRNA	Tissues sampled	FXTAS Onset	Co-morbid diagnoses	MRI findings	Age at death	Cause of death	FMR1 + relatives
Case 1	80	2.59 ± 0.42	Adrenal Testes Pineal	69	Cognitive decline Neuropathy Choking	Brain atrophy White matter disease	79	Aspiration pneumonia	1 grandchild with FXS PM daughter
Case 2	160	Not determined	Colon Testes	64	Seizures COPDHistory of smoking Type II diabetes Emphysema Depression Myelodysplastic syndrome with sideroblastic anemia, Cognitive decline Obsessive compulsive disorder Anxiety	White matter disease	69		4 grandchildren with FXS
Case 3	75	2.02 ± 0.27	Adrenal Colon Testes Pituitary	69	Dementia, Herpes zoster	Not determined	93	Aspiration pneumonia Pulmonary edema	Grandchildren with FXS 3 PM daughters
Case 4	85	4.25 ± 0.21	Adrenal Colon Heart Pituitary Pancreas	67	Hypertension Neuropathy Cognitive decline	Global brain atrophy MCP sign White matter disease	78		
Case 5	72	Not determined	Adrenal Testes Epididymis	80	Neuropathy Dementia Cardiac arrhythmia Hypertension Angina Type II diabetes Irritability	White matter disease in the pons	87	Stroke with hemiparesis	
Case 6	109	Not determined	Heart Kidney Pancreas Thyroid	63	Hypertension COPD Cardiac arrhythmia Cognitive decline Type II diabetes Pacemaker Syncope	Global brain atrophy White matter disease	73	Postoperative Carotid endarterectomy	2 grandchildren with FXS Premutation daughter
Case 7	95	3.35 ± 0.27	Esophagus Heart	56	Type II diabetes Cognitive decline Impotence Bladder incontinence Seizures Neuropathy Hypertension Dementia	Pituitary cyst Global brain atrophy Diffuse white matter disease	67	Myocardial infarction	Grandchildren with FXS

FXTAS Case	CGG	FMR1 mRNA	Tissues sampled	FXTAS Onset	Co-morbid diagnoses	MRI findings	Age at death	Cause of death	FMR1 + relatives
					Hallucination Mood lability				
Case 8	30, 96 AR = 0.76	2.80± 0.12	Mitral valve biopsy	49	PTSD Depression Hypertension Neuropathy Nonepileptic seizures Conversion disorder MVP Mitral regurgitation Hypothyroidism Anxiety	Mild brain atrophy, White matter disease	N/A	N/A	
Case 9	80	3.75 ± 0.51	Testes	65	Constipation Neuropathy Impotence Hypertension Type II diabetes Hyperlipidemia Cardiac arrythmia Pituitary adenoma Adenocarcinoma		72	Adenocarcinoma	
Case 10	95	Not determined	Pituitary	61	Impotence Hypertension Stroke Sleep apnea Mood lability Neuropathy Anxiety Prostate cancer Chronic pain Choking Irritability	Global brain atrophy White matter disease	67	Pulmonary edema Congestive heart failure	

FMR1 mRNA levels were not determined for Cases 2, 5, 6, 10 due to the unavailability of unfixed tissues. Activation ratio (AR: ratio of cells with the normal X as the active X) is provided for female Case 8

Table 2

Intranuclear inclusions are present in somatic organs and autonomic ganglia in human FXTAS cases and the CGG KI mouse model of the PM, organized by organ system and cell type

	Human FXTAS[a]	Cell type(s) with inclusions	CGG KI	Cell type(s) with inclusions
Adrenals	1, 3, 4, 5	Chromaffin secreting cells of medulla Steroid secreting cells of cortex	Yes	Chromaffin secreting cells of medulla
Colon	2, 3, 4	Ganglion cells of myenteric plexus & submucosal plexus	Yes	Ganglion cells of myenteric plexus
Esophagus	7	Ganglion cells of myenteric plexus	Not analyzed	
Heart	4, 6, 7	Cardiomyocytes ganglia	Yes	Cardiomyocytes
		Autonomic ganglia		Intracardiac autonomic ganglia
Mitral valve	8	Ganglion cells	Yes	Ganglion cells
		Smooth muscle cells		
Kidney	1, 6	Mesangial cells	None detected	-
Peripheral autonomic ganglia	4	Ganglion cells	Yes	Ganglion cells
Pituitary gland	3, 10	Acidophils	Yes	Pars anterior
		Basophils		Pars intermedia
		Pituicytes		
Testes	1, 2, 3, 5, 9	Leydig cells	None detected	–
Epididymis	5	Epithelial cells of distal tubule	Not analyzed	–
Pancreas	4, 6	Islets of Langerhans	Yes	Islets of Langerhans
				A and D cells
Thyroid	1, 6	Follicular Cells	Yes	Parafollicular cells
		Parafollicular cells		
Pineal gland	1	Ganglion cells	Yes	Ganglion Cells
		Pinealocytes		Pinealocytes
		Astroglia		Astroglia

[a] The number of organ tissues available for this series varied case by case. Intranuclear inclusions were present in all cases where that tissue was received (e.g., for the adrenals, 4 cases had adrenal tissue available, and intranuclear inclusions were seen in each of the 4 cases). See text for percentages of inclusions in each organ system