Title

Cerebral Microbleeds in Fragile X—Associated Tremor/Ataxia Syndrome

Journal

Movement Disorders, 36(8)

ISSN

0885-3185

Authors

Salcedo-Arellano, María Jimena

Wang, Jun Yi

McLennan, Yingratana A

et al.

Publication Date

2021-08-01

DOI

10.1002/mds.28559

Peer reviewed

HHS Public Access

Author manuscript

Mov Disord. Author manuscript; available in PMC 2024 March 12.

Published in final edited form as:

Mov Disord. 2021 August ; 36(8): 1935–1943. doi:10.1002/mds.28559.

Cerebral microbleeds in Fragile X-associated Tremor/Ataxia Syndrome

María Jimena Salcedo-Arellano, MD[1,2,3,4], **Jun Yi Wang, PhD**[2,5], **Yingratana A McLennan, BS**[1,2,3], **Mai Doan, BS**[3,4], **Ana Maria Cabal-Herrera, MD**[6], **Sara Jimenez, BS**[3,4], **Marisol W Wolf-Ochoa, BS**[3,4], **Desiree Sanchez, BS**[3,4], **Pablo Juarez, BS**[3,4], **Flora Tassone, PhD**[2,7], **Blythe Durbin-Johnson, PhD**[8], **Randi J Hagerman, MD**[1,2], **Verónica Martínez-Cerdeño, PhD**[2,3,4]

[1]Department of Pediatrics, University of California Davis School of Medicine, Sacramento, CA, USA

[2]Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, University of California Davis, Sacramento, CA, USA

[3]Institute for Pediatric Regenerative Medicine and Shriners Hospitals for Children Northern California, Sacramento, CA, USA

[4]Department of Pathology and Laboratory Medicine, UC Davis School of Medicine, Sacramento, CA, USA

[5]Center for Mind and Brain, University of California Davis, Davis, CA, USA

[6]Group on Congenital Malformations and Dysmorphology, Faculty of Health, Universidad del Valle (MACOS), Cali, Colombia

Corresponding author: Verónica Martínez-Cerdeño, Address: Shriners Hospitals for Children, 2425 Stockton BLVD, Sacramento CA 95817, USA, vmartinezcerdeno@ucdavis.edu.

Authors' Roles

MJSA – Conception, organization and execution of the research project (review of medical histories, staining, microscopic and image analysis). Writing of the first draft of the manuscript.
JYW – Conception of the research project.Writing of the first draft of the manuscript.
YAM - Execution of the research project (tissue preparation, staining, microscopic analysis and imaging). Writing of the first draft of the manuscript.
MD - Execution of the research project (tissue preparation, staining, microscopic analysis and imaging).
AMCH – Execution of the research project (statistical analysis). Writing of the first draft of the manuscript
SJ - Execution of the research project (tissue preparation, staining and imaging).
MWWO- Execution of the research project (tissue preparation & staining).
DS - Execution of the research project (tissue preparation, staining and imaging).
PJ – Execution of the research project (tissue preparation).
FT - Execution of the research project (CGG sizing).
BDJ - Execution of the research project (statistical analysis). Writing of the first draft of the manuscript.
RJH - Conception and organization of the research project. Review and critique of the manuscript.
VMC - Conception, organization and execution of the research project. Writing of the first draft of the manuscript. Review and critique of the manuscript.

Disclosure statement: RJH has received funding from Zynerba, Ovid and the Azrieli Foundation for carrying out treatment studies in patients with fragile X syndrome (FXS). She has also consulted with Fulcrum, Ovid and Zynerba regarding treatment studies in individuals with FXS. FT has received funds from Asuragen and Zynerba for studies in FXS and associated disorders. VMC has consulted with Paxmedica, and received funding from Zynerba for organization of conferences in FXS and associated disorders.

Financial Disclosures

VMC consults for PaxMedica

[7]Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Sacramento, CA, USA

[8]Division of Biostatistics, Department of Public Health Sciences, UC Davis School of Medicine, Sacramento, CA, USA

Abstract

Background: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease of late-onset developed by carriers of the premutation in the fragile x mental retardation 1 (*FMR1*) gene. Pathological features of neurodegeneration in FXTAS include toxic levels of *FMR1* messenger RNA (mRNA), ubiquitin-positive intranuclear inclusions, white matter disease, iron accumulation, and a proinflammatory state.

Objective: To analyze the presence of cerebral microbleeds (CMBs) in the brain of patients with FXTAS and investigate plausible causes for CMBs in FXTAS.

Methods: We collected cerebral and cerebellar tissue from 15 FXTAS cases and 15 control cases carrying *FMR1* normal alleles. We performed hematoxylin and eosin (H&E), Perls and Congo red stains; ubiquitin and amyloid β-protein (Aβ) immunostaining. We quantified the number of CMBs, amount of iron, presence of Aβ within the capillaries, and number of endothelial cells containing intranuclear inclusions. We evaluated the relationships between pathological findings using correlation analysis.

Results: We found intranuclear inclusions in the endothelial cells of capillaries and an increased number of CMBs in the brains of those with FXTAS, both of which are indicators of cerebrovascular dysfunction. We also found a suggestive association between the amount of capillaries that contain Aβ in the cerebral cortex and the rate of disease progression.

Conclusion: We propose microangiopathy as a pathologic feature of FXTAS.

Keywords

FXTAS; *FMR1* premutation; neurodegeneration; cerebral microbleeds

Introduction

Fragile X-associated tremor/ataxia syndrome

Fragile X-associated tremor/ataxia syndrome (FXTAS) was described 20 years ago in a series of cases of *FMR1* gene premutation male carriers (expansion between 55 and 200 CGG trinucleotide repeats) presenting with intention tremor and cerebellar ataxia (1, 2). The clinical presentation is variable and as the disease progresses the patients experience worsening tremor, cerebellar ataxia, cognitive decline, peripheral neuropathy and parkinsonism (3, 4). The age of clinical diagnosis is generally between 55 and 65 years old (5); being more prevalent in males (~40%) than in females (~16%) (6, 7). Females typically present with milder symptoms, less white matter (WM) hyperintensities (WMHs) on MRI, and slower progression (8).

The neurotoxicity induced by the increased levels of *FMR1* mRNA is the main trigger of its pathogenesis (9, 10). Furthermore, the non-coding region of *FMR1* premutation mRNA has been proposed to be translated into multiple repeat associated non-AUG (RAN) peptides, including the FMRpolyG (11). The neurotoxicity of this peptide lies on its ability to disrupt the structure of the neuronal nuclear lamina (12). In addition, mitochondrial alterations and increased concentrations of intracellular calcium lead to an abnormal cellular response to DNA damage and increase neural vulnerably to reactive oxygen species (ROS) (13, 14). The presence of ubiquitin-positive intranuclear inclusions in neurons and astrocytes (15, 16) is the major pathologic criterion in the postmortem diagnosis of FXTAS (17). These inclusions have also been reported in Purkinje cells (18) and in non-nervous tissues (19). Other pathologic findings include WM disease (WMD) (16); increased iron deposition in the putamen, the Purkinje layer of the cerebellum, and the choroid plexus (20–22); and the presence of activated and senescent microglia (23).

Cerebral amyloid angiopathy

We recently observed CMBs in some cases of FXTAS during routine postmortem microscopic pathological examinations. Both cerebral amyloid angiopathy (CAA) and non-amyloid small vessel disease resulting from hypertension are known to underlie the appearance of CMBs. CAA-associated hemorrhages are superficial and due to the primary involvement of vessels in the cerebral cortex and meninges (24). CAA-Type 1 affects capillaries, leptomeningeal and cortical arterioles, venules, small arteries, and veins; it is 4-times more likely than CAA-Type 2 to be associated with apolipoprotein E (*APOE) ε4* (25). Patients with Alzheimer's Disease (AD) and concomitant moderate-to-severe CAA are considered at higher risk for lobar intracerebral hemorrhages (26, 27). Dementia is suggested to occur in about 21–50% of men with FXTAS and the frequency increases at latter stages of the disease (5, 6, 28, 29); nevertheless, the prevalence of coexisting FXTAS and AD is unknown. A previous case-control study conducted in two primary dementia populations including AD did not find a difference in the frequency of *FMR1* premutation expansion between groups (30). However, a postmortem case series in females with a definite diagnosis of FXTAS found that half of cases had concomitant AD pathology (29). Similar neuropathological studies have not been conducted in males with FXTAS. In addition, the presence of at least one *APOE ε4* allele may contribute as a genetic factor predisposing to the development of FXTAS (31). In the current study, we quantified the burden of CMBs and CAA and evaluated their relation in cases of FXTAS and controls.

Iron deposition

The gradual deposit of iron in the process of aging is well known (32–34). Increased levels of iron are present in the substantia nigra in Parkinson's disease (PD) (35, 36) and in the putamen, caudate, and the temporal grey matter (GM) in AD (37–40). The motor and cognitive deficits found in patients with multiple sclerosis (MS) are correlated to excessive iron deposition in the GM (41, 42). Our prior studies reported mild iron accumulation in the Purkinje layer and the dentate nucleus of the cerebellum in a subset of FXTAS cases (21) and to a greater degree in the putamen and choroid plexus (20, 22). We quantified the area occupied by iron bound to hemosiderin and examined the association of iron deposits, if any, with the presence of CMBs.

Materials and methods

Sample collection

Tissue was obtained from 15 males with FXTAS and 15 age and sex-matched control cases from the FXS/FXTAS brain repository at UC Davis (a node of the Hispanic-American Brain Bank for Neurodevelopmental Disorders - CENE). Specimens were obtained through consented autopsies with the approval of the institutional review board (IRB). FXTAS cases had symptoms for many years before death (mean 8 years, SD 6.7) and were clinically diagnosed based on the presence of intention tremor, cerebellar ataxia, parkinsonism, memory and executive function deficits, and confirmed with the presence of the *FMR1* premutation and postmortem ubiquitin-positive intranuclear inclusions in brain cells. Control tissue was obtained from subjects without any significant neurological history nor the premutation. The age of FXTAS cases ranged from 58 to 85 years (average 73.9 years). Control cases ranged from age 53 to 81 (average 68.8 years). The number of CGG repeats in the FXTAS group was between 67 and 119 repeats (additional data in table 1).

Histology

Fixed samples from cerebral cortex Brodmann areas (BA)8, BA9, BA24, and cerebellum were immersed in 20% sucrose (Fisher, USA) and embedded in optimal cutting temperature (OCT) compound (Fisher). Blocks were cut on a freezing microtome at 14 μm thickness.

CGG sizing

Genomic DNA was isolated from brain tissue using standard procedures (Trizol, Invitrogen). CGG repeat allele size was determined using PCR and Southern blot analysis (43, 44).

Cerebral microbleeds quantification

While performing routine microscopic analysis of FXTAS brain samples, we observed that many cases presented with CMBs (Figure 1). In order to assess if CMBs were a characteristic of FXTAS, we analyzed tissue from the cerebral cortex and cerebellum of 15 FXTAS cases and 15 control cases (Figure 1). A blinded investigator evaluated the presence of CMBs in cerebral and cerebellar sections at 10x in H&E stained tissue (yes/no) and classified the number of CMBs with the following scoring system: 0 = No CMBs; 1 = 1–2 small CMBs; 2 = 1 large or 3 small CMBs; 3= ≥ 2 large or > 3 small CMBs.

Perl's staining

We treated sections in 1:1 10% potassium ferrocyanide (Fisher) and 20% hydrochloric acid (Fisher) for 10 minutes at room temperature, counterstained with Nuclear Fast red (Ricca Chemical, USA), dehydrated with ethanol, cleared with xylene, and coverslipped with Permount (Fisher). Blue (Prussian blue) color confirmed the presence of iron bound to hemosiderin in ferric state.

Congo red staining

We stained tissue with the modified Puchtler's Congo red amyloid method. We treated sections with modified Weigert's iron hematoxylin for 10 seconds, washed and placed in

acid alcohol solution, followed by a filtered Congo red solution (C6767, Sigma-Aldrich) for 20 minutes, dehydrated in ethanol, cleared with xylene, and coverslipped with Permount (Fisher) (45). Congo red stains amyloid protein aggregates when in a compacted form (46). Under cross-polarized light, stained aggregates show an apple-green birefringence (47) (Figure 2).

Iron quantification

We analyzed the area occupied by positive Prussian blue stain using NIH ImageJ software. Images from every FXTAS and control case were captured at 20x (Keyence microscope) and merged in a single image. We randomly selected areas of 5.04 mm^2/field from the cerebrum and 3.17 mm^2/field from the cerebellum and segmented the colors using the *colour deconvultion* plugin developed by G. Landini (http://www.dentistry.bham.ac.uk/landinig/software/cdeconv/cdeconv.html). We recorded the total percentage area occupied by Prussian blue for each sample.

Grading systems for cerebral amyloid angiopathy

We used two complementary grading systems to characterize CAA. The first, established by Olichney and colleagues in 1995 (48), classifies amyloid based on a 5-level positivity grading system, where 0 = no Aβ-positive blood vessels, 1 = scattered Aβ positivity, 2 = strong, circumferential Aβ in some blood vessels, 3 = widespread, strong, circumferential Aβ, and 4 = same as grade 3 with dysphoric changes. The second one, developed by Vonsattel el al (49), grades CAA based on the degree of amyloid infiltration into the vessel wall, mild = amyloid restricted to the tunica media, moderate = the tunica media is thicker than normal and replaced by amyloid, and severe = extensive deposition of amyloid, wall fragmentation, and leakage of blood through the vessel wall.

Ubiquitin and amyloid immunohistochemistry (IHC)

We incubated sections in DIVA for 8 minutes at 110°C followed by 3% hydrogen peroxide, permeabilized and blocked in a TBS based solution containing Triton and donkey serum for 1 hour (75% TBS, 15% Triton, 10% serum), incubated with primary antibodies overnight at 4 °C in a dark and humid box (rabbit anti-ubiquitin (1:150, Dako, Denmark) and polyclonal rabbit anti-β Amyloid 1–42 (ab10148, 1:100, Abcam)). On day 2, sections were incubated with donkey anti-rabbit biotinylated secondary antibody (1:150, Jackson) for 1 hour, incubated in Vectastain ABC kit (Vector Labs) for 2 hours, developed in DAB kit (Vector Labs), dehydrated with alcohols, cleared in xylene, and cover-slipped.

Quantification of ubiquitin-positive intranuclear inclusions

For the visualization of ubiquitin-positive intranuclear inclusions in endothelial cells two independent investigators quantified the first 20 capillaries encountered in the cerebral and cerebellar WM and GM. We report the average of the two totals. We identified brain capillaries by their histological characteristics, a thin wall composed of continuous endothelial lining supported by a basement membrane and less than 8 μm in diameter suitable for the passage of a single circulating blood cell (50). Brain capillaries were

characterized by the absence of astrocytic fibers, pericytes, nor smooth muscle cells. We took images on an Olympus microscope using 20X-100X oil objectives.

Statistical analysis

Statistical significance was defined as $P < 0.005$, per Benjamin and Berger (51), with $P < 0.05$ considered suggestive. Age was compared between FXTAS and control cases using a Wilcoxon rank-sum test, and categorical variables were compared between groups using Fisher's Exact Test. The associations between CMBs (which were ordered categorical variables) and group or clinical variables were analyzed using proportional odds logistic regression (52). The relationships between inclusions and CMBs or between years to progression and CMBs were analyzed using linear regression. Years to disease progression was log transformed prior to analysis in order to more closely satisfy model assumptions. Olichney scores were compared between groups and associated with clinical variables using proportional odds logistic regression. Vonsattel scores were compared between groups using Fisher's exact test, and associated with clinical variables using logistic regression. Iron accumulation was compared between groups and associated with clinical variables using linear models. Iron percentage was log transformed in order to more closely satisfy model assumptions. Analyses were conducted using R version 4.06.2 (2020–06-22) (53).

Results

Cerebral microbleeds are common in FXTAS

All FXTAS brains had WM alterations in the cerebral and the cerebellar cortex, including WM vacuolization, axonal loss and widened perivascular spaces, consistent with the histological definition of WMD. We quantified the number of CMBs in the GM and WM of the prefrontal cerebral cortex and cerebellum of FXTAS and control cases (Figure 1A–D).

We found that 56% of the FXTAS cases presented with CMBs in the WM of the cerebral and cerebellar cortices. The GM of the cerebral cortex was affected with CMBs in 40% of the cases, while 33% had CMBs in the GM of the cerebellum. In contrast, 6.6% control cases had CMBs in cerebral WM, 20% in cerebral GM, and 6.6% in cerebellar GM and WM. FXTAS cases presented with a significant increase in the number of CMBs in the cerebral WM ($P = 0.000891$) and in the cerebellar WM ($P = 0.000822$) compared to control cases (Table 2). The difference of CMB scores in cerebellar GM (OR 7.54, 95% CI 1.01–156.27, $P = 0.0483$) suggested that FXTAS cases had higher odds of a greater number of CMBs than control cases. We did not find a significant difference in the cerebral GM. In addition, we did not find any association between a higher CMB score in the cerebral WM, cerebral GM or cerebellar WM and age, number of CGG repeats, diagnosis of hypertension or stroke, use of anticoagulants or antiplatelets, and age of onset of FXTAS symptoms. However, data suggested that more years of disease progression might be associated with lower odds of a higher CMB score in the GM of the cerebellum ($P = 0.00657$). In other words, patients with FXTAS presenting with rapid disease progression had higher odds of a higher CMB score in the cerebellar GM (see supplementary material).

Iron accumulation in FXTAS

We visualized iron in the GM and WM of the prefrontal cerebral cortex and cerebellum using Perl's staining. There was no significant difference in iron occupancy between FXTAS and control cases across all of the studied brain regions. There was no association between the area occupied by iron in the cerebral and cerebellar cortices of FXTAS cases and CGG repeats, age, rate of disease progression (in years), diagnosis of hypertension, stroke, and anticoagulant use.

Cerebral amyloid angiopathy in FXTAS

We quantified the amount of amyloid contained in blood vessels in sections from BA8, BA9 and cerebellar cortex stained with Congo red and Aβ IHC (Figure 2) in FXTAS and control cases. We found that FXTAS cases had higher odds of a higher CAA-Olichney score than control cases in the prefrontal cerebral cortex (OR 4.44, 95% CI 1.02,22.00, $P = 0.0466$). We did not find a difference in the cerebellar cortex. Data suggest that a later onset of symptoms ($P = 0.0457$) and a faster progression of FXTAS before death ($P = 0.0199$) are associated with higher odds of a higher CAA-Olichney score in the cerebral cortex. Within FXTAS cases, while 35% had a mild and 28% a moderate Vonsattel-CAA score, but none had a severe score. In contrast, only mild CAA-Vonsattel scores were registered in 35% of the controls. The difference in CAA-Vonsattel score between groups was not statistically significant.

Intranuclear inclusions in cerebral endothelial cells

Endothelial cells stained with an antibody against ubiquitin and Nissl presented with a pale violet cytoplasm, a dark violet nucleus and brown intranuclear inclusions (Figure 3). In FXTAS, only some capillaries presented endothelial cells with inclusions, and only some endothelial cells contained an inclusion within a given capillary (mean 13%, SD 12%). The percentage of capillaries compromised with intranuclear inclusions in their endothelial cells showed high variability from case to case (from 0% to 35%), and each case also had variable presentation across different brain areas. Higher CMB scores in the cerebral prefrontal cortical WM ($P = 0.00598$) and GM ($P = 0.0201$) were suggestively associated with a higher percentage of capillaries with inclusions. No association was found in the cerebellar cortex.

Discussion

Cerebral microbleeds are expressions of cerebrovascular dysfunction in FXTAS

CMBs are microscopic bleedings associated with microangiopathy. The presence of CMBs in healthy subjects displays age-related increase in prevalence, ranging from 6.5% in 45–50 years old individuals to 35.7% in individuals aged 80 years (77). CMBs have a high prevalence in neurodegenerative diseases (78, 79) and chronic systemic hypertension (80). We found that most CMBs in FXTAS contained intact erythrocytes, suggesting that these were acute and not chronic CMBs. CMBs in the cortex of the human brain have been associated with the severe accumulation of Aβ in the walls of leptomeningeal and cortical small vessels (82), defined as CAA (83, 84). CAA is found in ~50–80% of patients with dementia (85, 86) and up to 95% of patients with AD (87, 88). We found higher CAA

scores in FXTAS relative to controls in the cerebral cortex but not in the cerebellum. This suggests that the presence of CAA in patients with FXTAS may contribute to a vascular component of pathology. However, we did not confirm a correlation between Olichney or Vonsattel-CAA scoring systems and CMB scores in FXTAS. Based on our analysis we cannot conclude that a grater number of CMBs is associated with more severe CAA in the studied brain regions of FXTAS cases. Our data are consistent with an AD's study reporting a CAA correlation with age and the severity of amyloid plaques deposition, while the correlation with microinfarcts was not statistically significant (84). Further studies are needed to examine additional causes of vascular pathology in FXTAS.

Iron deposits are located in specific brain regions in FXTAS

Abnormal iron accumulation can occur as part of the normal aging process, and in neurodegenerative diseases, such as MS, AD and CAA (90). Our previous work indicate that iron accumulated in the putamen, choroid plexus and dentate nucleus of the cerebellum in FXTAS (20–22). However, we did not find a generalized increase in the amount of iron deposits within the FXTAS cases in the cerebellar and prefrontal cerebral cortex (21). These data also suggest that the CMBs we describe in FXTAS are mostly acute, since chronic CMBs are associated with the deposition of blood-breakdown products, in particular iron bound to hemosiderin, within perivascular macrophages, rarely detected in the studied areas of our FXTAS cases.

Endothelial cells have intranuclear inclusions in FXTAS

We showed that endothelial cells in FXTAS present with intranuclear inclusions. We hypothesize that endothelial cells containing intranuclear inclusions in FXTAS are compromised, producing changes in the endothelial structure that may cause the discontinuation of the contact between cells. This is supported by the observation of widened perivascular spaces, which may be an indication of compromised blood-brain barrier. This alteration could translate into the breakage of the capillary and/or the dysfunction of tight junctions leading to increased vascular permeability and leakage of erythrocytes. Another possibility is that vascular changes are due to an inflammatory process that results in endothelial failure and neurovascular unit dysfunction (91, 92). FXTAS presents with a generalized proinflammatory state evident by the presence of microglial activation and microglial senescence (23), iron deposition (20–22), oxidative stress (93, 94) and elevated cytokine levels (95). Most likely, a combination of events above, in addition to coexisting CAA, is responsible for the capillary dysfunction that underpins the presence of CMBs in FXTAS.

Microangiopathy is a component of FXTAS

We consider FXTAS as a neurodegenerative disease associated with microangiopathy of the brain. Microangiopathy refers to conditions with damaged capillaries and characterized by CMBs, WMHs on MRI, reduced WM integrity, axonal injury, neuronal apoptosis, demyelination, oligodendrocyte damage, enlarged perivascular spaces, and brain atrophy (91, 96, 97), all presenting in FXTAS. Additionally, cerebrovascular dysfunction is linked to dementia, psychiatric disorders, and gait abnormalities, also characteristics of FXTAS (98, 99). Although features of microangiopathy have been reported in previous studies (60,

69), the current study demonstrates for the first time the evidence for a microangiopathy component in FXTAS pathology.

Limitations

Our data on postmortem analysis show that CMBs are common in the brain cortical WM in FXTAS. We have to be conservative with our conclusions given the studied sample size and variance of the measures. Some of the findings which were not significant during our evaluation might be due to the study being under-powered. We should also note that our findings relate only to the studied areas: prefrontal and cerebellar cortices. Additional studies are required to evaluate the burden of CMBs in other areas of the brain, including subcortical areas.

Conclusions

We demonstrated the presence of CMBs and intranuclear inclusions in endothelial cells in FXTAS. There is an increased number of CMBs in the cerebral and cerebellar cortical WM of FXTAS compared to control cases and a possible association between CMB scores and the percentage of capillaries with intranuclear inclusions in the endothelial cells of the cerebral cortex. There is also a positive association between the number of capillaries containing Aβ in the cerebral prefrontal cortex of FXTAS cases and the age of onset of symptoms and the rate of disease progression. We conclude that the underlying pathogenic mechanism of FXTAS compromises cerebral vasculature leading to a series of complex pathological changes and endothelial abnormalities. We suggest that the presence of CMBs in the cortical WM should be considered as part of the histopathologic manifestation of FXTAS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

The authors acknowledge the great contribution from the donors and their families to the field of neurodegenerative disorders, especially for the understanding of the neuropathology of FXTAS.

Funding:

This research was supported by funds from the National Institute of Neurological Disorders and Stroke (NINDS) grant R01 1NS107131, the National Institute of Child Health and Human Development (NICHD) grant R01 HD036071, the MIND Institute Intellectual and Developmental Disabilities Research Center P50 HD103526, the National Center for Advancing Translational Sciences, National Institute of Health, through grant UL1 TR001860, and Shriners Hospitals for Children – Northern California.

References

1. Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology. 2001;57(1):127–30. [PubMed: 11445641]
2. Hagerman RJ, Hagerman P. Fragile X-associated tremor/ataxia syndrome - features, mechanisms and management. Nat Rev Neurol. 2016;12(7):403–12. [PubMed: 27340021]

3. Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet. 2003;72(4):869–78. [PubMed: 12638084]
4. Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, et al. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). J Neurol Sci. 2006;248(1–2):227–33. [PubMed: 16780889]
5. Bourgeois JA, Cogswell JB, Hessl D, Zhang L, Ono MY, Tassone F, et al. Cognitive, anxiety and mood disorders in the fragile X-associated tremor/ataxia syndrome. Gen Hosp Psychiatry. 2007;29(4):349–56. [PubMed: 17591512]
6. Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA. 2004;291(4):460–9. [PubMed: 14747503]
7. Rodriguez-Revenga L, Madrigal I, Pagonabarraga J, Xuncla M, Badenas C, Kulisevsky J, et al. Penetrance of FMR1 premutation associated pathologies in fragile X syndrome families. Eur J Hum Genet. 2009;17(10):1359–62. [PubMed: 19367323]
8. Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, et al. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. Am J Hum Genet. 2004;74(5):1051–6. [PubMed: 15065016]
9. Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet. 2000;66(1):6–15. [PubMed: 10631132]
10. Berman RF, Buijsen RA, Usdin K, Pintado E, Kooy F, Pretto D, et al. Mouse models of the fragile X premutation and fragile X-associated tremor/ataxia syndrome. J Neurodev Disord. 2014;6(1):25. [PubMed: 25136376]
11. Todd PK, Oh SY, Krans A, He F, Sellier C, Frazer M, et al. CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. Neuron. 2013;78(3):440–55. [PubMed: 23602499]
12. Sellier C, Buijsen RAM, He F, Natla S, Jung L, Tropel P, et al. Translation of Expanded CGG Repeats into FMRpolyG Is Pathogenic and May Contribute to Fragile X Tremor Ataxia Syndrome. Neuron. 2017;93(2):331–47. [PubMed: 28065649]
13. Song G, Napoli E, Wong S, Hagerman R, Liu S, Tassone F, et al. Altered redox mitochondrial biology in the neurodegenerative disorder fragile X-tremor/ataxia syndrome: use of antioxidants in precision medicine. Molecular medicine (Cambridge, Mass.). 2016;22:548–59. [PubMed: 27385396]
14. Robin G, López JR, Espinal GM, Hulsizer S, Hagerman PJ, Pessah IN. Calcium dysregulation and Cdk5-ATM pathway involved in a mouse model of fragile X-associated tremor/ataxia syndrome. Human molecular genetics. 2017;26(14):2649–66. [PubMed: 28444183]
15. Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, et al. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain. 2002;125(Pt 8):1760–71. [PubMed: 12135967]
16. Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, et al. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain. 2006;129(Pt 1):243–55. [PubMed: 16332642]
17. Ma L, Herren AW, Espinal G, Randol J, McLaughlin B, Martinez-Cerdeno V, et al. Composition of the Intranuclear Inclusions of Fragile X-associated Tremor/Ataxia Syndrome. Acta Neuropathol Commun. 2019;7(1):143. [PubMed: 31481131]
18. Ariza J, Rogers H, Monterrubio A, Reyes-Miranda A, Hagerman PJ, Martinez-Cerdeno V. A Majority of FXTAS Cases Present with Intranuclear Inclusions Within Purkinje Cells. Cerebellum. 2016;15(5):546–51. [PubMed: 27108270]
19. Greco CM, Soontrapornchai K, Wirojanan J, Gould JE, Hagerman PJ, Hagerman RJ. Testicular and pituitary inclusion formation in fragile X associated tremor/ataxia syndrome. J Urol. 2007;177(4):1434–7. [PubMed: 17382748]

20. Ariza J, Rogers H, Hartvigsen A, Snell M, Dill M, Judd D, et al. Iron accumulation and dysregulation in the putamen in fragile X-associated tremor/ataxia syndrome. Mov Disord. 2017;32(4):585–91. [PubMed: 28233916]
21. Rogers H, Ariza J, Monterrubio A, Hagerman P, Martinez-Cerdeno V. Cerebellar Mild Iron Accumulation in a Subset of FMR1 Premutation Carriers with FXTAS. Cerebellum. 2016;15(5):641–4. [PubMed: 27259564]
22. Ariza J, Steward C, Rueckert F, Widdison M, Coffman R, Afjei A, et al. Dysregulated iron metabolism in the choroid plexus in fragile X-associated tremor/ataxia syndrome. Brain Res. 2015;1598:88–96. [PubMed: 25498860]
23. Martínez-Cerdeño V, Hong T, Amina S, Lechpammer M, Ariza J, Tassone F, et al. Microglial cell activation and senescence are characteristic of the pathology FXTAS. Movement Disorders. 2018;In press.
24. Mehndiratta P, Manjila S, Ostergard T, Eisele S, Cohen ML, Sila C, et al. Cerebral amyloid angiopathy-associated intracerebral hemorrhage: pathology and management. Neurosurg Focus. 2012;32(4):E7.
25. Thal DR, Ghebremedhin E, Rub U, Yamaguchi H, Del Tredici K, Braak H. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol. 2002;61(3):282–93. [PubMed: 11895043]
26. van Veluw SJ, Charidimou A, van der Kouwe AJ, Lauer A, Reijmer YD, Costantino I, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. Brain. 2016;139(Pt 12):3151–62. [PubMed: 27645801]
27. Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM. Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. Ann Neurol. 2001;49(6):697–705. [PubMed: 11409420]
28. Juncos JL, Lazarus JT, Graves-Allen E, Shubeck L, Rusin M, Novak G, et al. New clinical findings in the fragile X-associated tremor ataxia syndrome (FXTAS). Neurogenetics. 2011;12(2):123–35. [PubMed: 21279400]
29. Tassone F, Greco CM, Hunsaker MR, Seritan AL, Berman RF, Gane LW, et al. Neuropathological, clinical and molecular pathology in female fragile X premutation carriers with and without FXTAS. Genes Brain Behav. 2012;11(5):577–85. [PubMed: 22463693]
30. Hall DA, Bennett DA, Filley CM, Shah RC, Kluger B, Ouyang B, et al. Fragile X gene expansions are not associated with dementia. Neurobiol Aging. 2014;35(11):2637–8. [PubMed: 24958193]
31. Silva F, Rodriguez-Revenga L, Madrigal I, Alvarez-Mora MI, Oliva R, Mila M. High apolipoprotein E4 allele frequency in FXTAS patients. Genet Med. 2013;15(8):639–42. [PubMed: 23492875]
32. Drayer B, Burger P, Darwin R, Riederer S, Herfkens R, Johnson GA. MRI of brain iron. AJR Am J Roentgenol. 1986;147(1):103–10. [PubMed: 3487201]
33. Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. J Neurochem. 1958;3(1):41–51. [PubMed: 13611557]
34. Thomas LO, Boyko OB, Anthony DC, Burger PC. MR detection of brain iron. AJNR Am J Neuroradiol. 1993;14(5):1043–8. [PubMed: 8237678]
35. Berg D, Hochstrasser H. Iron metabolism in Parkinsonian syndromes. Mov Disord. 2006;21(9):1299–310. [PubMed: 16817199]
36. Gorell JM, Ordidge RJ, Brown GG, Deniau JC, Buderer NM, Helpern JA. Increased iron-related MRI contrast in the substantia nigra in Parkinson's disease. Neurology. 1995;45(6):1138–43. [PubMed: 7783878]
37. Bartzokis G, Sultzer D, Cummings J, Holt LE, Hance DB, Henderson VW, et al. In vivo evaluation of brain iron in Alzheimer disease using magnetic resonance imaging. Arch Gen Psychiatry. 2000;57(1):47–53. [PubMed: 10632232]
38. Bartzokis G, Sultzer D, Mintz J, Holt LE, Marx P, Phelan CK, et al. In vivo evaluation of brain iron in Alzheimer's disease and normal subjects using MRI. Biol Psychiatry. 1994;35(7):480–7. [PubMed: 8018799]

39. House MJ, St Pierre TG, Kowdley KV, Montine T, Connor J, Beard J, et al. Correlation of proton transverse relaxation rates (R2) with iron concentrations in postmortem brain tissue from alzheimer's disease patients. Magn Reson Med. 2007;57(1):172–80. [PubMed: 17191232]
40. House MJ, St Pierre TG, Foster JK, Martins RN, Clarnette R. Quantitative MR imaging R2 relaxometry in elderly participants reporting memory loss. AJNR Am J Neuroradiol. 2006;27(2):430–9. [PubMed: 16484425]
41. Brass SD, Benedict RH, Weinstock-Guttman B, Munschauer F, Bakshi R. Cognitive impairment is associated with subcortical magnetic resonance imaging grey matter T2 hypointensity in multiple sclerosis. Mult Scler. 2006;12(4):437–44. [PubMed: 16900757]
42. Tjoa CW, Benedict RH, Weinstock-Guttman B, Fabiano AJ, Bakshi R. MRI T2 hypointensity of the dentate nucleus is related to ambulatory impairment in multiple sclerosis. J Neurol Sci. 2005;234(1–2):17–24. [PubMed: 15993137]
43. Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, et al. A novel FMR1 PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem. 2010;56(3):399–408. [PubMed: 20056738]
44. Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. J Mol Diagn. 2008;10(1):43–9. [PubMed: 18165273]
45. Center UoRM;Pageshttps://www.urmc.rochester.edu/urmc-labs/pathology/stainsmanual/index.html?MODIFIEDPUCHTLERCONGOREDAMYLOIDMETHOD12020.
46. Wilcock DM, Gordon MN, Morgan D. Quantification of cerebral amyloid angiopathy and parenchymal amyloid plaques with Congo red histochemical stain. Nat Protoc. 2006;1(3):1591–5. [PubMed: 17406451]
47. Howie AJ, Brewer DB, Howell D, Jones AP. Physical basis of colors seen in Congo red-stained amyloid in polarized light. Lab Invest. 2008;88(3):232–42. [PubMed: 18166974]
48. Olichney JM, Hansen LA, Hofstetter CR, Grundman M, Katzman R, Thal LJ. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol. 1995;52(7):702–8. [PubMed: 7619027]
49. Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP, Jr. Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. Ann Neurol. 1991;30(5):637–49. [PubMed: 1763890]
50. Practical Surgical Neuropathology: A Diagnotic Approach. Second ed: Elsevier; 2018.
51. Benjamin DJ, Berger JO. Three Recommendations for Improving the Use of p-Values. The American Statistician. 2019;73(sup1):186–91.
52. Agresti A. Categorical Data Analysis. Second ed: John Wiley & Sons, Inc; 2002.
53. Team RC 2019;Pageshttps://www.R-project.org/ on November 11, 2019.
54. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: improved N3 bias correction. IEEE Trans Med Imaging. 2010;29(6):1310–20. [PubMed: 20378467]
55. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. Neuroimage. 2006;31(3):1116–28. [PubMed: 16545965]
56. Li W, Avram AV, Wu B, Xiao X, Liu C. Integrated Laplacian-based phase unwrapping and background phase removal for quantitative susceptibility mapping. NMR Biomed. 2014;27(2):219–27. [PubMed: 24357120]
57. Wei H, Gibbs E, Zhao P, Wang N, Cofer GP, Zhang Y, et al. Susceptibility tensor imaging and tractography of collagen fibrils in the articular cartilage. Magn Reson Med. 2017;78(5):1683–90. [PubMed: 28856712]
58. Ashburner J, Friston K. Rigid body registration. In: Frackowiak R, Friston K, Frith C, et al., eds. Human Brain Function. 2 ed: Academic Press; 2003.
59. Hall DA, Birch RC, Anheim M, Jønch AE, Pintado E, O'Keefe J, et al. Emerging topics in FXTAS. Journal of neurodevelopmental disorders. 2014;6(1):31-. [PubMed: 25642984]
60. Apartis E, Blancher A, Meissner WG, Guyant-Marechal L, Maltete D, De Broucker T, et al. FXTAS: new insights and the need for revised diagnostic criteria. Neurology. 2012;79(18):1898–907. [PubMed: 23077007]

61. Kalus S, King J, Lui E, Gaillard F. Fragile X-associated tremor/ataxia syndrome: An under-recognised cause of tremor and ataxia. J Clin Neurosci. 2016;23:162–4. [PubMed: 26439425]
62. Gouw AA, Seewann A, van der Flier WM, Barkhof F, Rozemuller AM, Scheltens P, et al. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. J Neurol Neurosurg Psychiatry. 2011;82(2):126–35. [PubMed: 20935330]
63. Tassone F, Hall D. FXTAS, FXPOI, and Other Premutation Disorders. Second ed. New York City: Springer International Publishing Switzerland; 2016.
64. Leehey MA. Fragile X-Associated Tremor/Ataxia Syndrome. Journal of Investigative Medicine. 2009;57(8):830. [PubMed: 19574929]
65. Filley CM. Fragile X tremor ataxia syndrome and white matter dementia. The Clinical Neuropsychologist. 2016;30(6):901–12. [PubMed: 27356088]
66. Hall DA, Hermanson M, Dunn E, Stebbins G, Merkitch D, Ouyang B, et al. The Corpus Callosum Splenium Sign in Fragile X-Associated Tremor Ataxia Syndrome. 2017;4(3):383–8.
67. Adams JS, Adams PE, Nguyen D, Brunberg JA, Tassone F, Zhang W, et al. Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS) Neurology. 2007;69(9):851–9. [PubMed: 17724287]
68. Wang JY, Hessl DH, Hagerman RJ, Tassone F, Rivera SM. Age-dependent structural connectivity effects in fragile x premutation. Arch Neurol. 2012;69(4):482–9. [PubMed: 22491193]
69. Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, et al. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. AJNR Am J Neuroradiol. 2002;23(10):1757–66. [PubMed: 12427636]
70. Famula JL, McKenzie F, McLennan YA, Grigsby J, Tassone F, Hessl D, et al. Presence of Middle Cerebellar Peduncle Sign in FMR1 Premutation Carriers Without Tremor and Ataxia. Front Neurol. 2018;9:695. [PubMed: 30186228]
71. Tassone F, Hall DA. FXTAS, FXPOI, and Other Premutation Disorders. Second ed. Ney York City: Springer International Publishing AG Switzerland; 2016.
72. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70(1):9–14. [PubMed: 11118240]
73. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28(3):652–9. [PubMed: 9056627]
74. Hocking DR, Loesch DZ, Trost N, Bui MQ, Hammersley E, Francis D, et al. Total and regional white matter lesions are correlated with motor and cognitive impairments in carriers of the FMR1 premutation. Front Neurol. 2019;10:Article 832.
75. Nandigam RN, Viswanathan A, Delgado P, Skehan ME, Smith EE, Rosand J, et al. MR imaging detection of cerebral microbleeds: effect of susceptibility-weighted imaging, section thickness, and field strength. AJNR Am J Neuroradiol. 2009;30(2):338–43. [PubMed: 19001544]
76. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, et al. Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. AJNR Am J Neuroradiol. 1999;20(4):637–42. [PubMed: 10319975]
77. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, et al. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. Stroke. 2010;41(10 Suppl):S103–6. [PubMed: 20876479]
78. Lesnik Oberstein SA, van den Boom R, van Buchem MA, van Houwelingen HC, Bakker E, Vollebregt E, et al. Cerebral microbleeds in CADASIL. Neurology. 2001;57(6):1066–70. [PubMed: 11571335]
79. Dichgans M, Holtmannspotter M, Herzog J, Peters N, Bergmann M, Yousry TA. Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. Stroke. 2002;33(1):67–71. [PubMed: 11779891]

80. Kidwell CS, Saver JL, Villablanca JP, Duckwiler G, Fredieu A, Gough K, et al. Magnetic resonance imaging detection of microbleeds before thrombolysis: an emerging application. Stroke. 2002;33(1):95–8. [PubMed: 11779895]
81. Shoamanesh A, Kwok CS, Benavente O. Cerebral microbleeds: histopathological correlation of neuroimaging. Cerebrovasc Dis. 2011;32(6):528–34. [PubMed: 22104448]
82. Thal DR. The pre-capillary segment of the blood-brain barrier and its relation to perivascular drainage in Alzheimer's disease and small vessel disease. ScientificWorldJournal. 2009;9:557–63. [PubMed: 19578713]
83. Soontornniyomkij V, Lynch MD, Mermash S, Pomakian J, Badkoobehi H, Clare R, et al. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. Brain Pathol. 2010;20(2):459–67. [PubMed: 19725828]
84. Kovari E, Herrmann FR, Hof PR, Bouras C. The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013;39(5):498–509. [PubMed: 23163235]
85. Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. Neurology. 1996;46(6):1592–6. [PubMed: 8649554]
86. Pfeifer LA, White LR, Ross GW, Petrovitch H, Launer LJ. Cerebral amyloid angiopathy and cognitive function: the HAAS autopsy study. Neurology. 2002;58(11):1629–34. [PubMed: 12058090]
87. Greenberg SM, Briggs ME, Hyman BT, Kokoris GJ, Takis C, Kanter DS, et al. Apolipoprotein E epsilon 4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy. Stroke. 1996;27(8):1333–7. [PubMed: 8711797]
88. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. J Neural Transm (Vienna). 2002;109(5–6):813–36. [PubMed: 12111471]
89. Rosand J, Muzikansky A, Kumar A, Wisco JJ, Smith EE, Betensky RA, et al. Spatial clustering of hemorrhages in probable cerebral amyloid angiopathy. Ann Neurol. 2005;58(3):459–62. [PubMed: 16130107]
90. Amaral LLF, Gaddikeri S, Chapman PR, Roy R, Gaddikeri RS, Marussi VH, et al. Neurodegeneration with Brain Iron Accumulation: Clinicoradiological Approach to Diagnosis. 2015;25(4):539–51.
91. Hakim AM. Small Vessel Disease. Front Neurol. 2019;10:1020. [PubMed: 31616367]
92. Shoamanesh A, Preis SR, Beiser AS, Vasan RS, Benjamin EJ, Kase CS, et al. Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham Heart Study. Neurology. 2015;84(8):825–32. [PubMed: 25632086]
93. Ross-Inta C, Omanska-Klusek A, Wong S, Barrow C, Garcia-Arocena D, Iwahashi C, et al. Evidence of mitochondrial dysfunction in fragile X-associated tremor/ataxia syndrome. Biochem J. 2010;429(3):545–52. [PubMed: 20513237]
94. Giulivi C, Napoli E, Tassone F, Halmai J, Hagerman R. Plasma metabolic profile delineates roles for neurodegeneration, pro-inflammatory damage and mitochondrial dysfunction in the FMR1 premutation. Biochem J. 2016;473(21):3871–88. [PubMed: 27555610]
95. Dufour BD, Amina S, Martinez-Cerdeno V. FXTAS presents with upregulation of the cytokines IL12 and TNFalpha. Parkinsonism Relat Disord. 2020;82:117–20. [PubMed: 33285358]
96. Rosano C, Marsland AL, Gianaros PJ. Maintaining brain health by monitoring inflammatory processes: a mechanism to promote successful aging. Aging Dis. 2012;3(1):16–33. [PubMed: 22500269]
97. Rosano C, Watson N, Chang Y, Newman AB, Aizenstein HJ, Du Y, et al. Aortic pulse wave velocity predicts focal white matter hyperintensities in a biracial cohort of older adults. Hypertension. 2013;61(1):160–5. [PubMed: 23172923]
98. Kim HJ, Kang SJ, Kim C, Kim GH, Jeon S, Lee JM, et al. The effects of small vessel disease and amyloid burden on neuropsychiatric symptoms: a study among patients with subcortical vascular cognitive impairments. Neurobiol Aging. 2013;34(7):1913–20. [PubMed: 23414669]
99. Pinter D, Ritchie SJ, Doubal F, Gattringer T, Morris Z, Bastin ME, et al. Impact of small vessel disease in the brain on gait and balance. Sci Rep. 2017;7:41637. [PubMed: 28134332]

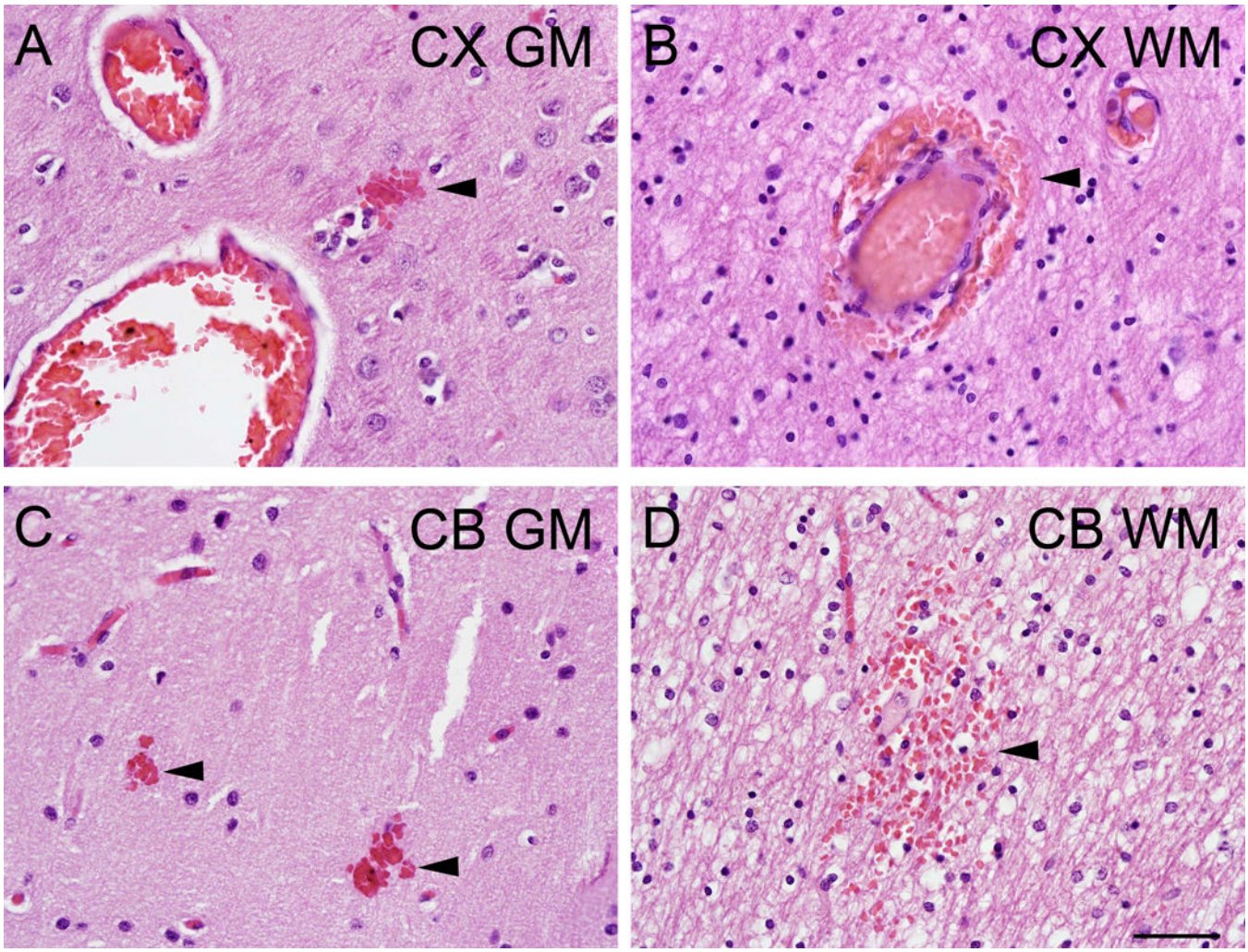

Figure 1.
Cerebral microbleeds (CMBs) stained with H&E. CMBs in WM and GM of (A-B) prefrontal cortex and (C-D) cerebellum. Scale bar: 50 μm.

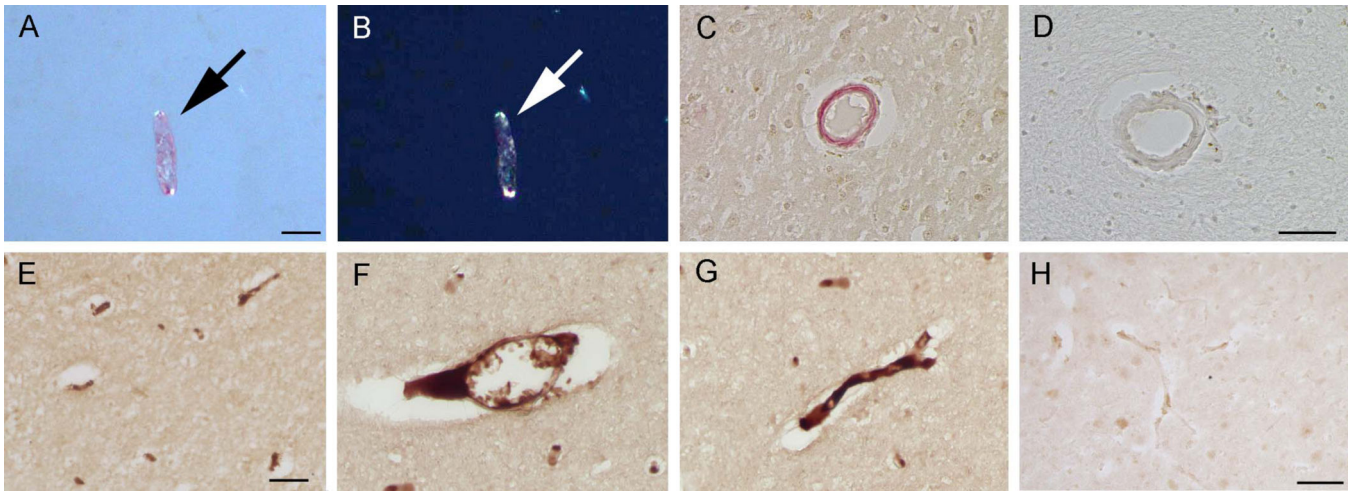

Figure 2.

Aβ in blood vessels. (A-B) Aβ in blood vessel in WM of the prefrontal cortex in FXTAS, stained with congo red under polarized light. (C-D) Aβ in blood vessel in WM of the prefrontal cortex, stained with congo red under brightfield. (C) Aβ blood vessel in WM of prefrontal cortex in FXTAS. (D) Negative Aβ blood vessel in WM in a control case. (E-H) Aβ blood vessels in prefrontal cortex stained with an antibody against Aβ. (E-G) Aβ blood vessels in prefrontal cortex WM in FXTAS. (H) Negative Aβ blood vessel in prefrontal cortex WM in a control case. Scale bar in A-B: 25 μm; C-D: 20 μm; E: 20 μm; F-H: 10 μm.

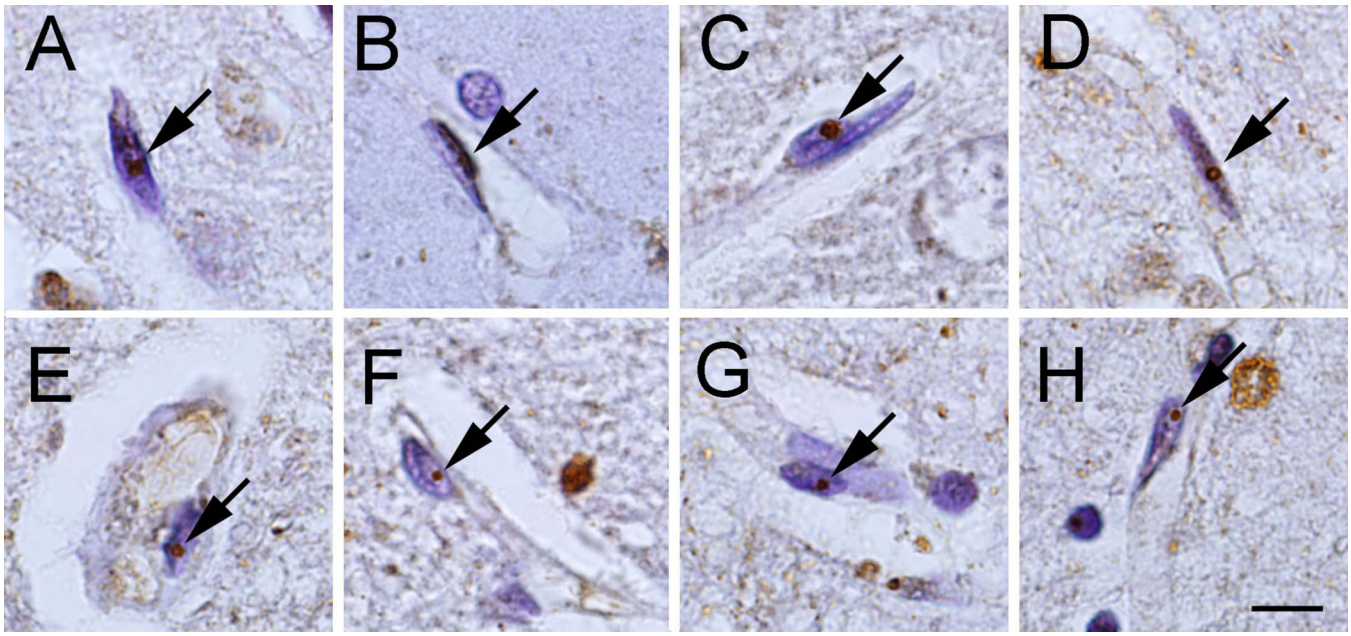

Figure 3.

Intranuclear inclusionsions in capillaries. (A-H) Ubiquitin stained intranuclear inclusions (brown) in endothelial cells of capillaries in the prefrontal cortex of FXTAS cases. Endothelial cell nuclei are elongated and stained in purple with cresyl violet. Scale bar: 10 μm.

Table 1.

Subject Characteristics by Group

	Control (n = 15)	Case (n = 15)	All Subjects (n = 30)	P-Value
Age (Years)				0.1008
N	15	15	30	
Mean (SD)	68.8 (8.8)	73.9 (7.9)	71.4 (8.6)	
Median (Range)	68 (53−−81)	75 (58−−85)	71.5 (53−−85)	
Anticoagulant Meds				0.2926
None	3 (20%)	5 (33.3%)	8 (26.7%)	
Aspirin	1 (6.7%)	6 (40%)	7 (23.3%)	
Warfarin	2 (13.3%)	1 (6.7%)	3 (10%)	
Unknown	9 (60%)	3 (20%)	12 (40%)	
Stroke				0.0752
No	5 (33.3%)	13 (86.7%)	18 (60%)	
Yes	5 (33.3%)	2 (13.3%)	7 (23.3%)	
Unknown	5 (33.3%)	0	5 (16.7%)	
HTN				0.1760
No	1 (6.7%)	6 (40%)	7 (23.3%)	
Yes	8 (53.3%)	8 (53.3%)	16 (53.3%)	
Unknown	6 (40%)	1 (6.7%)	7 (23.3%)	

Table 2.

Proportional Odds Logistic Regression Models of Brain CMBs in Cases and Controls

	Odds Ratio (95% Confidence Interval)	P-Value
Cerebral cortex-WM	21.8 (3.13, 451.19)	0.000891
Cerebral cortex-GM	2.82 (0.59, 16.10)	0.197
Cerebellar cortex-WM	22.2 (3.19, 458.70)	0.000822
Cerebellar cortex-GM	7.54 (1.01, 156.27)	0.0483