



Research article

Imaging of aluminium and amyloid β in neurodegenerative diseaseChristopher Exley^{*}, Matthew J. Mold

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ABSTRACT

Objectives: Recent research has confirmed the presence of aluminium in human brain tissue. Quantitative analyses suggest increased brain aluminium content in a number of neurodegenerative diseases including familial Alzheimer's disease, congophilic amyloid angiopathy, epilepsy and autism. Complementary aluminium-specific fluorescence microscopy identifies the location of aluminium in human brain tissue and demonstrates significant differences in distribution between diseases. Herein we combine these approaches in investigating associations between aluminium in human brain tissue and specific disease-associated neuropathologies.

Methods: We have used aluminium-specific fluorescence microscopy, Congo red staining using light and polarised light and thioflavin S fluorescence microscopy on serial sections of brain tissues to identify co-localisation of aluminium and amyloid β and tau neuropathology.

Results: A combination of light, polarised and fluorescence microscopy demonstrates an intimate relationship between aluminium and amyloid β in familial Alzheimer's disease but not in other conditions and diseases, such as congophilic amyloid angiopathy and autism. We demonstrate preliminary evidence of amyloid β pathology, including associations with vasculature and parenchymal tissues, in autism in tissues heavily loaded with aluminium.

Conclusion: We suggest that complementary aluminium-specific fluorescence microscopy may reveal important information about the putative toxicity of aluminium in neurodegenerative and neurodevelopmental disorders.

1. Introduction

Human exposure to aluminium is increasing [1]. A burgeoning body burden of an element that is only inimical to human health must be a concern [2]. The Aluminium Age has rendered human exposure as inevitable (<https://www.hippocraticpost.com/mens-health/the-aluminium-age/>). The workplace is no exception and in some circumstances may represent an accelerated exposure to aluminium [3, 4, 5]. The brain is a major target of aluminium intoxication [6, 7]. Aluminium is present in human brain tissue [8]. A burgeoning database documents its content in brain tissue from a variety of donors including those without discernible neuropathology to individuals who died with a recognised neurodegenerative disease or neurodevelopmental disorder [9]. Measurements of absolute amounts of aluminium in brain tissue have allowed the establishment of toxicity thresholds and confirmed long held understanding that its distribution is focal and likely associated with specific neuropathologies. The data document the presence of aluminium in all brain regions studied and, in general, do not identify any lobe-specific accumulations. However, these important quantitative determinations of

content are now complemented with aluminium-specific fluorescence microscopy [10] showing the exact location of aluminium in human brain tissue. When combined with standard microscopy methods to identify disease neuropathology such as amyloid β and tau these complementary data are proving useful and revealing in equating the presence of aluminium with its toxicity and potential contribution to disease aetiology. Herein we combine aluminium-specific fluorescence microscopy with other forms of light and fluorescence microscopy to reveal co-relationships between aluminium deposition and the neuropathology of a number of neurodegenerative diseases and neurodevelopmental disorders.

2. Materials and methods

2.1. Tissues

All tissues studied herein were provided as prepared slides by brain banks following ethical approval. Details of the latter are available in each of the cited source publications.

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2.2. Deparaffinisation and rehydration of brain tissue sections

All chemicals were purchased from Sigma Aldrich unless otherwise stated. Brain tissue sections were dewaxed using the d-limonene based reagent Histo-Clear (National Diagnostics, US) and rehydrated through an ethanol gradient into ultrapure water (conductivity <0.067 $\mu\text{S}/\text{cm}$). Rehydrated tissue sections were subsequently outlined with a hydrophobic PAP pen for staining, allowing for staining to be performed in humidity chambers using low reagent volumes (200 μL).

2.3. Lumogallion staining for the detection of aluminium

Rehydrated serial brain tissue sections were incubated in humidity chambers for 45 min in 1mM lumogallion (4-chloro-3-(2,4-dihydroxyphenylazo)-2-hydroxybenzene-1-sulphonic acid, TCI Europe N.V. Belgium) prepared in 50mM PIPES buffer (1,4-Piperazinediethanesulphonic acid), pH 7.4. Sections for autofluorescence were incubated in the buffer only and staining was performed at ambient temperature away from light. Following staining, sections were washed with fresh 50mM PIPES buffer, pH 7.4 and rinsed for 30s in ultrapure water. Sections were mounted with the aqueous mounting medium Fluoromount™ under glass coverslips.

2.4. Congo red staining for the detection of amyloid β

Rehydrated sections for Congo red staining were immersed in 0.5% w/v Congo red in 50% v/v ethanol for 5 min at ambient temperature. Sections were subsequently differentiated via immersion in 0.2% w/v potassium hydroxide prepared in 80% v/v ethanol for 3 s and washed for 30 s in ultrapure water. Stained sections were subsequently mounted using the aqueous mounting medium Faramount (Agilent Dako, UK) under glass coverslips.

2.5. Thioflavin S staining for the detection of amyloid β

Thioflavin S (ThS) staining was performed in humidity chambers via the addition of ca 0.075% w/v ThS, prepared in 50% v/v ethanol to rehydrated and PAP pen outlined brain tissue sections for 8 min. Staining was performed at ambient temperature away from light. Following staining, slides were immersed twice for 10 s in fresh solutions of 80% v/v ethanol. Sections were subsequently washed and agitated in ultrapure water for 30 s. Sections stained in this way were mounted with Fluoromount™.

2.6. Fluorescence and light microscopy

All microscopy was performed by use of an Olympus BX50 fluorescence microscope equipped with a vertical illuminator and BX-FLA reflected light attachment (mercury source). Lumogallion fluorescence and respective autofluorescence were captured using a U-MNIB3 fluorescence filter cube (excitation filter: 470–495nm, dichromatic mirror: 505nm, longpass barrier filter: 510nm). ThS fluorescence was captured using a U-MWBV2 filter cube (excitation filter: 400–440nm, dichromatic mirror: 455nm, longpass barrier filter: 475nm). All fluorescence filter cubes were from Olympus, UK. Congo red staining was visualised under brightfield illumination and polarised light using a U-POT polariser and a U-ANT transmitted light analyser (both from Olympus, UK). Images were acquired using a ColorView III CCD camera and the CellD software suite (Olympus, SiS Imaging Solutions, GmbH).

3. Results

3.1. Aluminium and cerebral amyloid angiopathy

An earlier observation of a high content of brain aluminium coincident with a rare form of sporadic early onset cerebral amyloid

angiopathy (CAA) suggested that amyloid β and aluminium might be co-localised in this tissue [11]. The case showed severe widespread congophilic angiopathy in cerebral cortical and leptomeningeal vessels. There were no senile plaques and few neurofibrillary tangles. CAA was widespread throughout brain tissue, with the exception of the hippocampus, as identified by positive Congo red staining and apple green birefringence under polarised light (Figure 1A). Congo red-positive spherulites under polarised light showed classical Maltese cross diffraction patterns and occasional apple-green birefringence (Figure 1B). While lumogallion fluorescence identified aluminium in all tissues examined, aluminium was not co-located with extracellular deposits of amyloid β [12]. Indeed the majority of aluminium deposits were intracellular and associated with glia-like cells including microglia and astrocytes (Figure 1C,D). Aluminium was in lymphocyte-like cells and cells lining the choroid plexus. Some deposits of aluminium were intraneuronal and there were a number of extracellular deposits of aluminium often surrounded by aluminium-loaded glia. Critically while amyloid β was unerringly extracellular and associated with vasculature aluminium deposits were almost exclusively intracellular.

3.2. Aluminium and epilepsy

We recently reported the first quantitative data on aluminium in human brain tissue in epilepsy [13]. The analyses showed high concentrations of aluminium in two areas of the brain, occipital lobe and hippocampus, known to be affected in epilepsy. Qualitative imaging by aluminium-specific fluorescence fully supported the quantitative data and especially in the hippocampus where a consistent observation was non-specific aluminium-rich cellular debris surrounded by aluminium-loaded glia-like cells (Figure 2A). Congo red failed to identify amyloid β in any tissue while ThS revealed intracellular and extracellular tau in neurofibrillary tangle-like deposits in all tissues though without evidence of co-localisation with aluminium (Figure 2B).

3.3. Aluminium and autism

While there are data for very few donors, the aluminium content of brain tissue in autism is invariably very high and especially in the light of the young age of donors [14]. However, the stand-out, and at the time unique, observation in autism brain tissues was the almost exclusive intracellular, non-neuronal location of aluminium deposits. While aluminium was found associated with extracellular, probably neuronal, debris as well as being located in some neuronal bodies it was primarily found as membrane-bound deposits in non-neuronal cells including microglia and lymphocytes (Figure 3A,B). In an attempt to relate observations of aluminium with potential neuropathology, we have identified amyloid β -like deposits in autism brain tissue and examined its potential relationship with aluminium. Future research using immunolabelling is required to confirm unequivocally that the observed deposits are amyloid β and not some other form of amyloid.

Amyloid β was positively identified using both Congo red and thioflavin S throughout donor tissues. A consistent observation was amyloid β , exhibiting apple-green birefringence under polarised light, associated with vasculature. Amyloid β was confirmed using both methods in the perivascular region of blood vessels and resembled classical cerebral amyloid angiopathy (Figure 3C,D). Parenchymal deposits of amyloid β , positive for both apple-green birefringence and ThS, were also observed in hippocampal tissues of donors (Figure 3E,F). While both amyloid β and aluminium were widely distributed throughout these brain tissues, there were very few instances where there was evidence of co-localisation.

3.4. Aluminium and familial Alzheimer's disease

Some of the highest concentrations of aluminium ever measured in human brain tissue were recorded in donors with a diagnosis of familial Alzheimer's disease (fAD) [15]. Initial investigations suggested that the

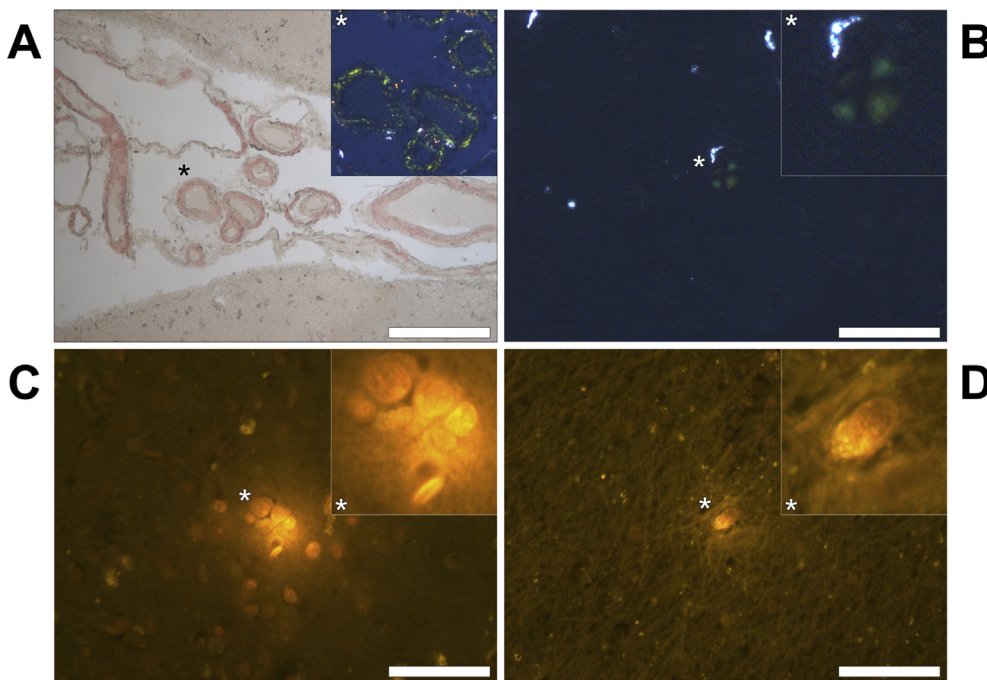


Figure 1. Amyloid β and aluminium deposition in brain tissue of a 59-year-old female donor with cerebral amyloid angiopathy (CAA). (A) Congo red reactive amyloid β was identified in leptomeningeal vessels in the parietal lobe by a red hue that produced apple-green birefringence under polarised light (magnified insert) and occasionally as spherulites as revealed by Maltese cross diffraction patterns, in the same lobe (B). Intracellular aluminium (orange) in microglia surrounding astrocytes (C) and appearing as punctate deposits within astrocytes (D), in cortical and white matter regions, respectively (asterisks denote magnified inserts). Figure adapted from [12] under CC license. Bars = (A): 200 μ m (B - D): 50 μ m.

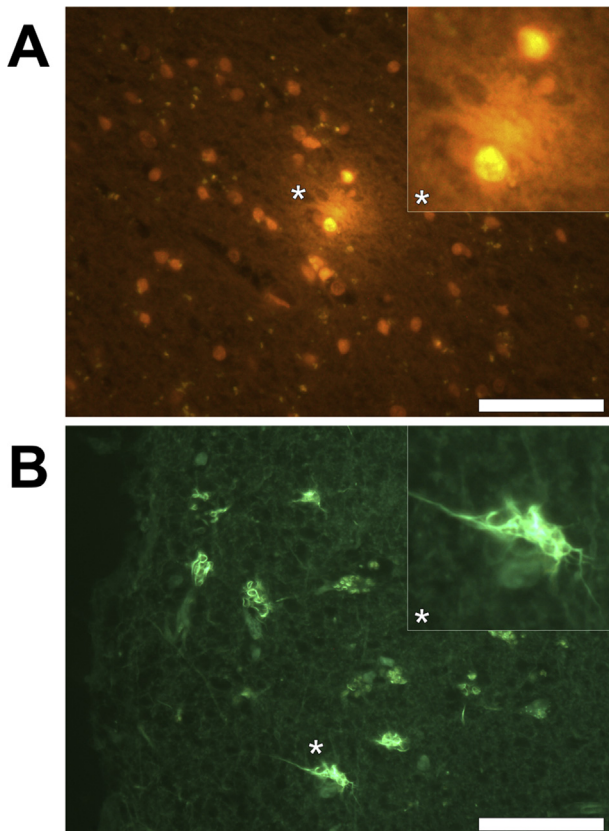


Figure 2. Intracellular aluminium and neurofibrillary tangles in brain tissue of a 60-year-old male donor with epilepsy. (A) Aluminium (orange) rich cellular debris surrounded by aluminium-loaded glial cells in the parahippocampal gyrus. (B) No lumogallion-reactive aluminium was identified in the parietal lobe and instead, cortical thioflavin S (ThS) positive neurofibrillary tangles (green) were observed (magnified inserts are denoted by asterisks). Figure adapted from [13] under CC license. Bars = 50 μ m.

majority of aluminium deposits were extracellular with a tentative suggestion of an association with β amyloid. A close relationship between aluminium and amyloid β in fAD is now confirmed with myriad examples throughout all donor tissues [16]. The co-localisation of aluminium and amyloid β in senile plaques was especially evident (Figure 4) and there were also examples of cerebral amyloid angiopathy exhibiting co-deposits of aluminium and apple-green birefringent positive amyloid β . ThS additionally identified neurofibrillary tangles in these tissues some in the immediate vicinity of aluminium-rich senile plaques.

4. Discussion

In fAD, where there is a genetic predisposition resulting in a higher body burden of amyloid β earlier in life [17], we observed intimate associations between deposits of aluminium and amyloid β in brain tissue [16]. Tissues from all four main lobes of the brain included many senile plaques and almost invariably, aluminium was co-located with these structures (Figure 4). The intimacy of the co-localisations in many cases suggested direct associations and even co-deposition of aluminium and amyloid β . However, such immediate relationships cannot be confirmed by microscopy. Cerebral amyloid angiopathy was also observed in many of these tissues and was similarly accompanied by deposits of aluminium. At first glance, these observations in fAD seem to present a *prima facie* case for the co-deposition of amyloid β and aluminium in fAD. However, in a case of aggressive, early onset CAA involving the exclusive deposition of amyloid β with the vasculature (no senile plaques) and very high levels of brain tissue aluminium there were no clear associations between these factors (Figure 1). Aluminium was not co-located with amyloid β in this case of early onset sporadic AD with extensive CAA [11, 12]. We also identified examples of CAA in autism brain tissue (Figure 3C,D) and while these tissues were heavily loaded with aluminium (Figure 3A,B), similarly to the aforementioned case of CAA we did not find evidence of co-localisation of aluminium and amyloid β in either the vasculature or other tissues in autism. In epilepsy, there was no evidence of amyloid β anywhere in the brain and so no relationship with aluminium could be tested. We have made the intriguing observation that in fAD where the majority of deposits of aluminium were extracellular there were close relationships between aluminium and amyloid β . However, in CAA and autism where the majority of deposits of aluminium were intracellular,

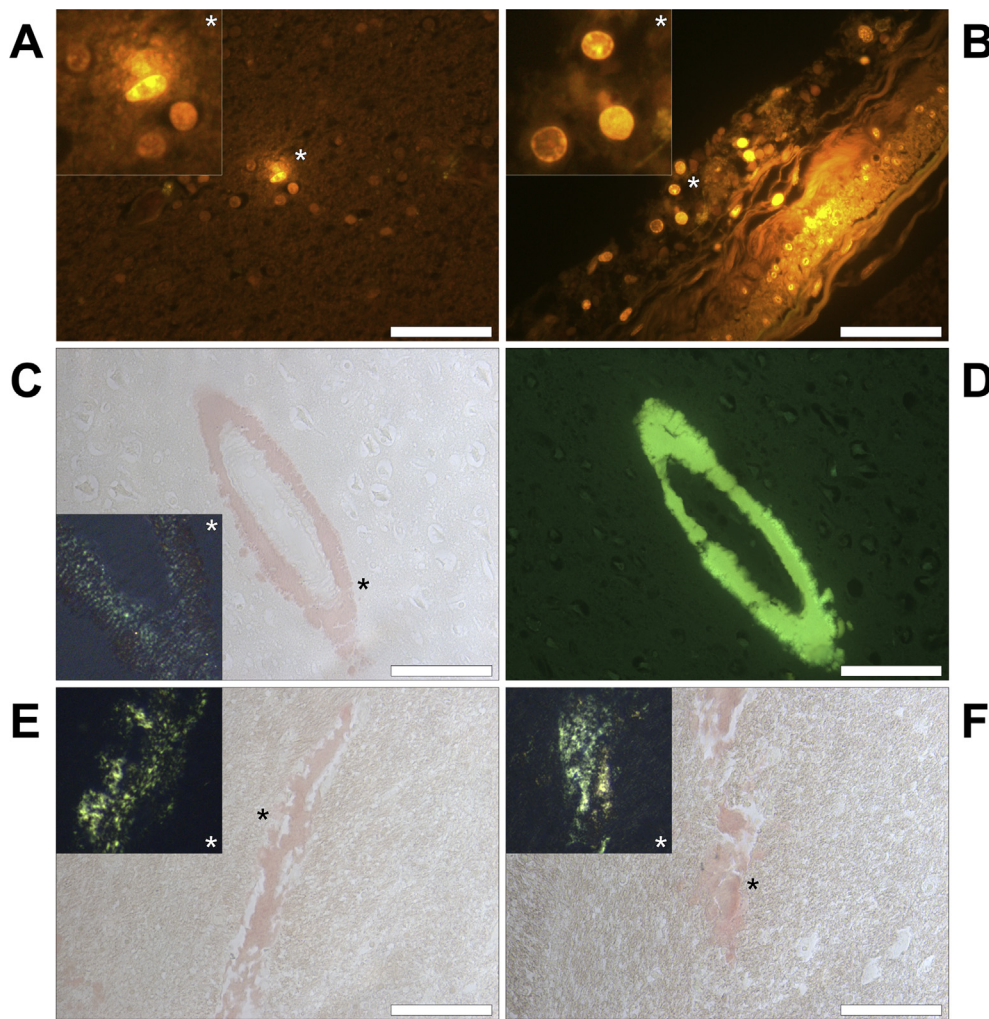


Figure 3. Intracellular aluminium, CAA and parenchymal amyloid β -like deposits in brain tissue of donors diagnosed with autism. (A) Aluminium-loaded (orange) microglial cell surrounding extracellular aluminium rich cellular debris in the temporal cortex of a 15-year-old male donor. (B) Intracellular aluminium in lymphocytes within leptomeningeal membranes in the hippocampus of a 50-year-old male donor. (C) Positive Congo red staining indicative of CAA in a cortical blood vessel in the frontal lobe a 14-year-old male donor. (D) Positive thioflavin S (ThS) staining (green) in the same cortical blood vessel. Parenchymal deposits of Congo red reactive amyloid in the hippocampus of (E) 33 and (F) 22-year-old male donors depicting apple-green birefringence in the highlighted (asterisks) regions when viewed under polarised light. Magnified inserts are denoted by asterisks. A & B adapted from [14] under CC license. Bars = (A & B): 50 μ m (C - F): 100 μ m.

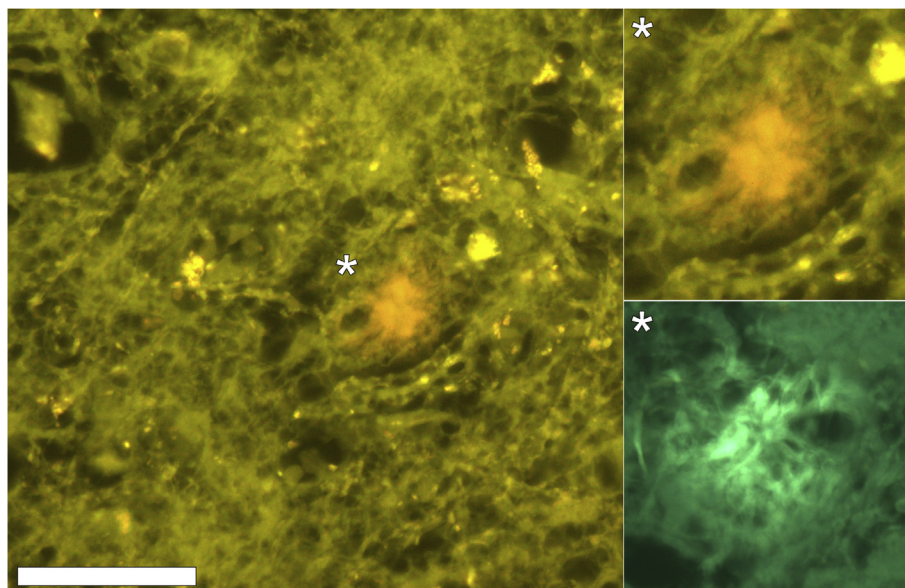


Figure 4. Aluminium co-localised with amyloid β in a senile plaque identified in the white matter of the parietal lobe of a donor diagnosed with familial Alzheimer's disease (fAD). Lumogallion-reactive aluminium was identified via an orange fluorescence emission, co-localised with positive thioflavin S (ThS) staining (green) in the lower magnified insert. Bar = 50 μ m.

aluminium was not associated with amyloid β in either the vasculature or parenchymal tissues. This raises the possibility that predispositions to an increased body burden of amyloid β are also predispositions to the co-localisation, perhaps even co-deposition, of aluminium and amyloid β in brain tissue. The comparison with pure CAA (amyloid β only associated with vasculature) and autism may also suggest different routes of entry of aluminium into brain tissue. For example, is a higher body burden of amyloid β facilitating the entry of aluminium into brain tissue in fAD. In pure CAA and autism, the preponderance of brain aluminium deposits in non-neuronal intracellular compartments including glia and lymphocytes suggests a role for diapedesis [18] in aluminium entry into brain tissue. There is a long history identifying interactions between aluminium and amyloid β , both *in vitro* and *in vivo*. The former support amyloid β binding aluminium [19, 20, 21] while the latter have proposed their co-localisation and especially in Alzheimer's disease [22, 23]. There are no data to confirm binding of aluminium (or any metal cation) by amyloid β *in vivo* (animal models or human) and the observations herein are the first to suggest that while the association of aluminium with amyloid β *in vivo* in humans is not inevitable it is possible, such as in fAD.

Our observations of amyloid β -like deposits in autism brain tissue are novel and may suggest neuropathology similar to that seen in CAA. We identified several examples of CAA in autism brain tissue as well as deposits of amyloid β in β sheet conformations in parenchymal tissues (Figure 3C-F). Previous research identified diffuse deposits of amyloid β in brain tissue in older individuals with autism (39 and 50 years of age) [24] while herein amyloid β in β sheet conformations was confirmed in individuals with autism aged, 14, 15, 22, 33, 44 and 50 years of age. Recently the non-amyloidogenic pathway of metabolism of amyloid precursor protein has been implicated in autism [25] while our observations suggest that the amyloidogenic pathway may also be important.

Tau pathologies and specifically neurofibrillary tangles (NFT) were identified using ThS in epilepsy (Figure 2) and in fAD. While historically aluminium has been shown to be co-located with tau in NFT we were unable to demonstrate the co-localisation of aluminium and tau using fluorescence microscopy.

Quantitative data demonstrating aluminium in brain tissues in neurodegenerative disease has been helpful in implicating aluminium in disease aetiology. Recent developments in aluminium-specific fluorescence microscopy have complemented quantitative data in providing information pertaining to the location of aluminium in human brain tissue. These novel observations now raise intriguing questions concerning the relationship between amyloid β and aluminium in neurodegenerative disease and suggest an intimate association in fAD.

Declarations

Author contribution statement

Christopher Exley: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Matthew J Mold: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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