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Toxicology

Aluminium in brain tissue in familial Alzheimer's disease

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ABSTRACT

The genetic predispositions which describe a diagnosis of familial Alzheimer's disease can be considered as cornerstones of the amyloid cascade hypothesis. Essentially they place the expression and metabolism of the amyloid precursor protein as the main tenet of disease aetiology. However, we do not know the cause of Alzheimer's disease and environmental factors may yet be shown to contribute towards its onset and progression. One such environmental factor is human exposure to aluminium and aluminium has been shown to be present in brain tissue in sporadic Alzheimer's disease. We have made the first ever measurements of aluminium in brain tissue from 12 donors diagnosed with familial Alzheimer's disease. The concentrations of aluminium were extremely high, for example, there were values in excess of 10 μg/g tissue dry wt. in 5 of the 12 individuals. Overall, the concentrations were higher than all previous measurements of brain aluminium except cases of known aluminium-induced encephalopathy. We have supported our quantitative analyses using a novel method of aluminium-selective fluorescence microscopy to visualise aluminium in all lobes of every brain investigated. The unique quantitative data and the stunning images of aluminium in familial Alzheimer's disease brain tissue raise the spectre of aluminium's role in this devastating disease.

1. Introduction

Genetic mutations associated with both the expression [1] and metabolism [2] of amyloid precursor protein (APP) are, in general, the basis for a diagnosis of familial Alzheimer's disease (fAD). They, along with evidence from Down's syndrome [3], provide strong support for the amyloid cascade hypothesis [4,5] and a central role for the neuropathology and biochemistry of amyloid-beta (Aβ) in Alzheimer's disease [6]. In many ways, familial AD has been used as a blueprint for understanding and treatment of sporadic or late-onset AD.

Aluminium is present in human brain tissue [7] and in a recent study involving 60 human brains the median aluminium content of 712 tissues across all four main lobes was 1.02 μg/g dry wt. with 75% of all values being <2.01 μg/g dry wt. tissue [8]. The association of aluminium and AD has a significant history [9,10] and yet there remains no consensus as to a role for this known neurotoxin in the disease [11]. However, recent reports concerning sporadic AD [12] and environmental [13] and occupational [14] exposure to aluminium have allowed the conclusion to be drawn that, under certain conditions, it is inevitable that aluminium will contribute towards AD [11,15]. The suggestion is made that wherever in the brain the concentration of aluminium is pathologically-concerning (>2.00 μg/g dry wt.) that this aluminium will contribute towards any ongoing AD and will result in the disease being earlier in onset with a more aggressive aetiology [15].

Familial AD is characterised by an earlier age of onset and yet there are no data to describe the aluminium content of brain tissue in this 'signature' form of AD. Herein we have obtained brain tissue from 12 autopsy-confirmed cases of familial AD and we have carried out the first ever measurements of brain aluminium content in familial AD. We have also supported our quantitative measurements with imaging of brain aluminium by aluminium-selective fluorescence microscopy.

2. Materials and methods

There was ethical approval from The MRC London Neurodegenerative Diseases Brain Bank at King's College, London (08/MRE09/38 + 5).

Samples of cortex of approximately 1 g frozen weight from temporal, frontal, parietal and occipital lobes were obtained from 12

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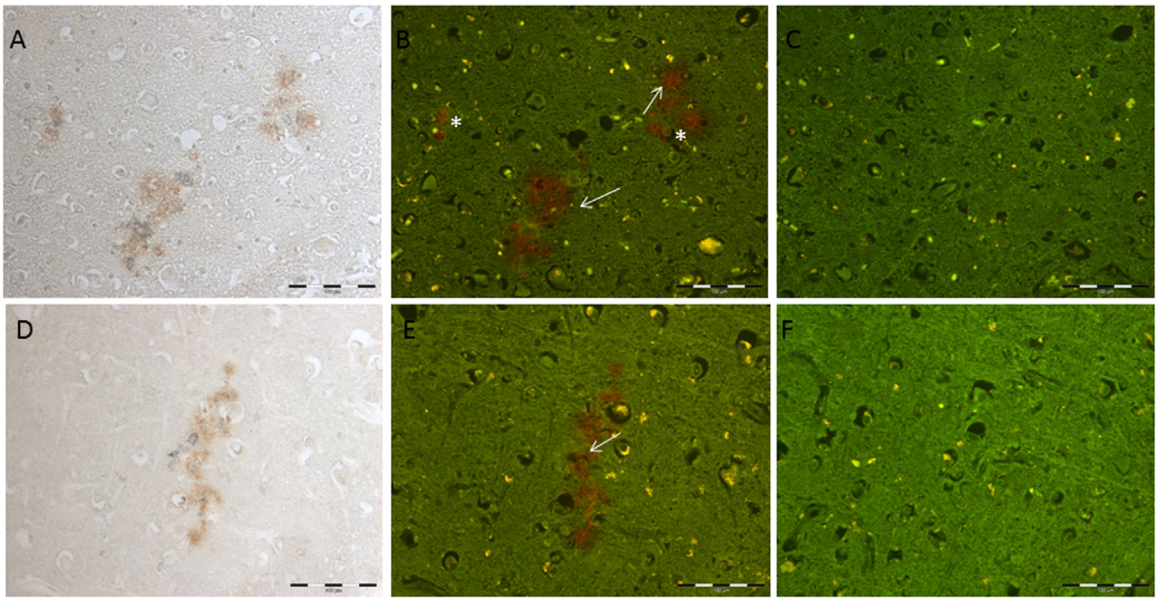

Fig. 1. Representative images of aluminium in frontal cortex. Light (A&D) and fluorescence (B&E) microscopy images of lumogallion-stained sections of frontal cortex. Asterisk label suggested intracellular deposits while arrows show diffuse deposits. Fluorescence microscopy of un-stained adjacent tissue sections (C&F) show autofluorescence. Scale bars are all 100 μm.

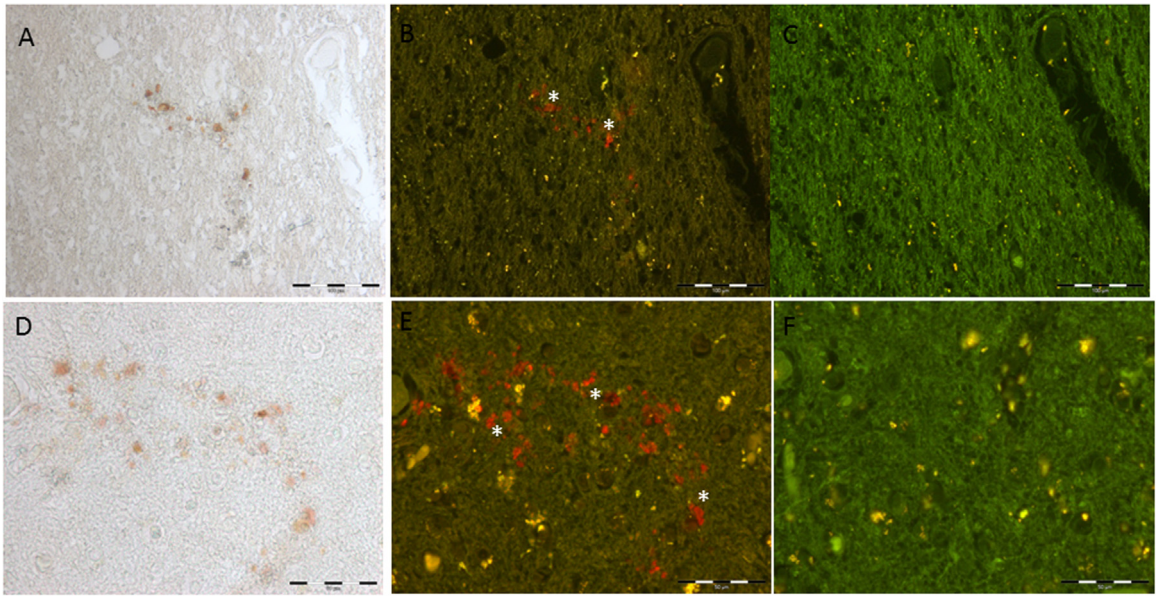

Fig. 2. Representative images of aluminium in parietal cortex. Light (A&D) and fluorescence (B&E) microscopy images of lumogallion-stained sections of frontal cortex. Asterisk label suggested intracellular deposits associated with both living and dead cells. Fluorescence microscopy of un-stained adjacent tissue sections (C&F) show autofluorescence. Scale bars are 100 μm (A–C) and 50 μm (D–F).

cases of autopsy-confirmed familial Alzheimer's disease. Donors with familial Alzheimer's disease are extremely rare and we had the privilege of obtaining tissue from the only 12 cases available at the brain bank with the year of diagnosis ranging from 1991 through to 2009. There were 7 female and 5 male donors in the age range 42–86. The bases for a diagnosis of familial Alzheimer's disease are given in Table 1. Where no definite genetic mutation was recorded at the time of autopsy we have defined probable familial Alzheimer's disease as either (i) having two first degree relatives with a dementing illness or (ii) where the patient or first degree relative had developed symptoms of dementia at less than 60 years of age or (iii) where the patient had one first and one second degree relative with dementia and one with symptoms of dementia at less than 60 years of age.

The aluminium content of these tissues was measured by an established and fully validated method [8] which herein is described briefly. Thawed tissues were cut using a stainless steel blade to give individual samples of *ca* 0.3 g (3 sample replicates for each lobe) wet weight and dried to a constant weight at 37 °C. Dried and weighed tissues were digested in a microwave (MARS Xpress CEM Microwave Technology Ltd.) in a mixture of 1 mL 15.8 M HNO_3 (Fisher Analytical Grade) and 1 mL 30% *w/v* H_2O_2 (BDH Aristar). Digests were clear with no fatty residues and, upon cooling, were made up to 5 mL volume using ultrapure water (cond <0.067 μS/cm). Total aluminium was measured in each sample

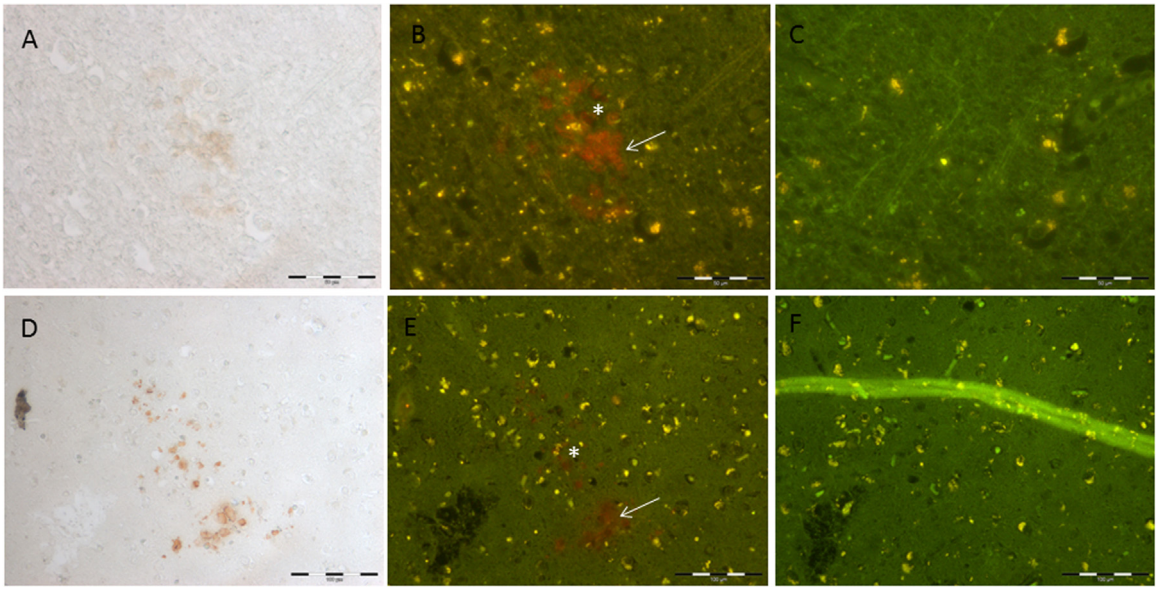

Fig. 3. Representative images of aluminium in cortex. Light (A&D) and fluorescence (B&E) microscopy images of lumogallion-stained sections of frontal (A&B) and temporal (D&E) cortex. Asterisk label suggested intracellular deposits and arrows diffuse deposits. Fluorescence microscopy of un-stained adjacent frontal (C) and temporal (F) tissue sections show autofluorescence. Scale bars are 50 μm (A–C) and 100 μm (D–F).

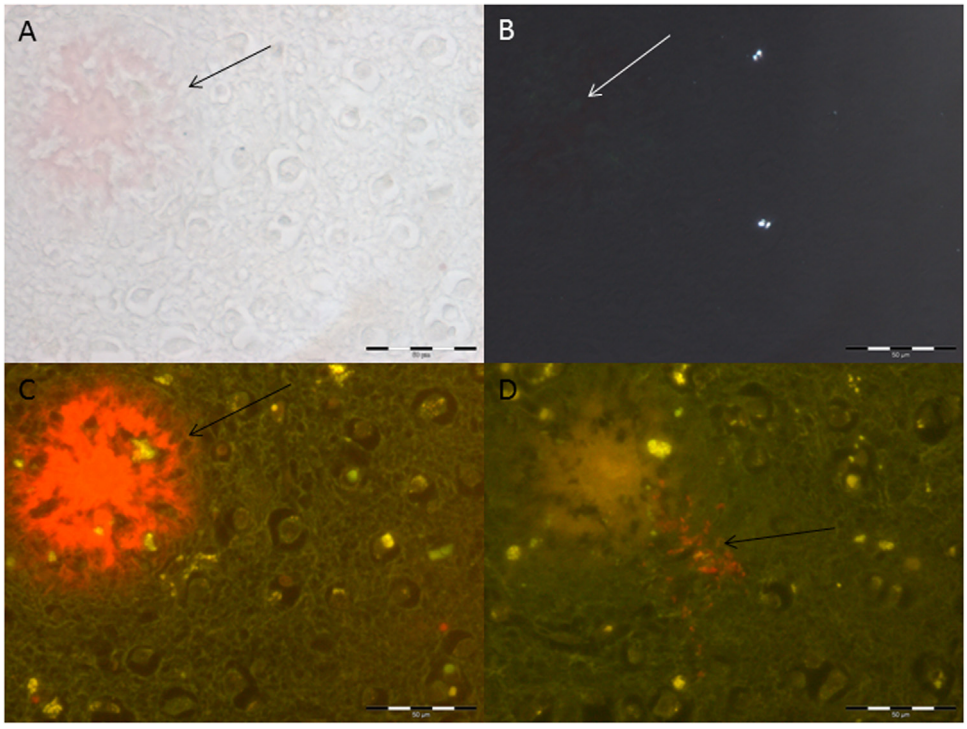

Fig. 4. Co-localisation of amyloid and aluminium in occipital cortex. (A) Light microscopy image of Congo red-stained tissue showing (arrow) senile plaque-like amyloid deposit. (B) Polarising microscopy image of Congo red-stained image showing (arrow) apple-green birefringence characteristic of amyloid in β sheet conformation. (C) Fluorescence microscopy image of Congo red-stained tissue showing (arrow) senile plaque-like amyloid deposit. (D) Fluorescence microscopy image of adjacent section of tissue stained with lumogallion and showing (arrow) significant deposits of aluminium. Scale bars are all 50 μm.

by transversely-heated graphite furnace atomic absorption spectrometry (TH GFAAS) using matrix-matched standards and an established analytical programme [8].

Formalin-fixed brain tissue for 6 cases of familial AD (A1, A5, A7, A8, A11 & A12) was supplied as pre-embedded paraffin blocks representing cortical tissue from frontal, parietal, and temporal and occipital lobes. A recently developed, optimised and validated method using lumogallion and fluorescence microscopy was used to identify the presence of aluminium in tissues [16]. This method was shown to be specific for aluminium with no interference from any other metals and no issues relating to autofluorescence. Slides of serial adjacent sections of tissue were imaged using an Olympus BX50 fluorescence microscope with a BX-FLA reflected light fluorescence attachment, equipped with a mercury burner and a vertical illuminator. For lumogallion (and autofluorescence) imaging a U-MNIB3 fluorescence filter cube was used (bandpass

excitation filter: 470–495 nm, dichromatic mirror: 505 nm, long-pass emission filter: 510 nm) (both from Olympus, UK). Images were obtained using Cell^{D} software (Olympus Soft Imaging Solutions GmbH).

3. Results

3.1. Aluminium content of brain tissues

Aluminium was found in all 144 tissues and its concentration ranged from 0.01 to 35.65 μg/g dry wt. (Table 1). The mean aluminium content for whole brains (n = 12) ranged from 0.34(0.26) for individual A1 to 6.55(9.59) μg/g dry wt. for individual A8. Approximately 40% of tissues (57/144) had an aluminium content considered as pathologically-concerning (≥2.00 μg/g dry wt.) while approximately 58% of these tissues had an aluminium content considered as pathologically-significant (≥3.00 μg/g dry wt.). The brains of 11 out of 12 individuals had at least one tissue with a pathologically-significant content of aluminium. The brains of 9 individuals had at least one tissue with an aluminium content ≥5.00 μg/g dry wt. while 5 of these had at least one tissue with an aluminium content ≥10.00 μg/g dry wt. (Table 1). The mean (SD)

Table 1
The content of aluminium in each of 4 lobes (O – occipital; F – frontal; T – temporal; P – parietal) of 12 cases of familial Alzheimer's disease. Values considered as pathologically-concerning or pathologically-significant are given in italics or bold typescript respectively. Details of pathology and known genetic mutations are also given. PSEN – presenilin, APP – amyloid precursor protein, BNE – Brain Net Europe.

Donor ID	Pathology and genetics	Gender	Age	Lobe	Replicate	[Al] μg/g dry wt.
A1	Braak/BNE stage VI	M	47	O	1	0.47
					2	0.67
					3	0.03
	Grandfather, father, brother died with dementia,brother had a PSEN-1 mutation			F	1	0.84
					2	0.52
					3	0.13
				T	1	0.16
					2	0.02
					3	0.29
				P	1	0.53
					2	0.28
					3	0.13
					Mean(SD)	0.34(0.26)
A2	Braak Stage VI	F	76	O	1	**10.12**
					2	**8.32**
	Father and nephew died with dementia, nephew developed dementia at young age				3	**11.54**
				F	1	1.75
					2	**4.48**
					3	0.48
				T	1	0.69
					2	0.55
					3	0.16
				P	1	0.27
					2	1.25
					3	1.60
					Mean(SD)	3.43(4.17)
A3	Braak Stage V-VI	M	53	O	1	*2.07*
					2	*2.83*
	Father died at young age with AD				3	**7.97**
				F	1	**3.23**
					2	1.55
					3	**3.93**
				T	1	0.70
					2	1.41
					3	*2.72*
				P	1	**3.18**
					2	*2.17*
					3	**5.32**
					Mean(SD)	3.09(1.97)
A4	Braak Stage V-VI	M	42	O	1	0.10
					2	**4.79**
	PSEN-1(DELTA4)				3	0.52
				F	1	**3.11**
					2	0.85
					3	1.48
				T	1	0.48
					2	*2.37*
					3	*2.51*
				P	1	**8.40**
					2	1.11
					3	0.39
					Mean(SD)	2.18(2.39)
A5	Braak Stage at least IV	M	60	O	1	0.59
					2	0.73
	Mother and brother died in their 50 s with dementia				3	0.14
				F	1	1.24
					2	**6.51**
					3	**13.41**
				T	1	0.77
					2	0.34
					3	0.25
				P	1	*2.61*
					2	0.41
					3	0.54
					Mean(SD)	2.30(3.93)
A6	Braak stage V-VI	F	86	O	1	**25.80**
					2	0.66
	Daughter died in 30 s with dementia				3	*2.25*
				F	1	**3.91**
					2	**9.89**
					3	1.61

Table 1 (*Continued*)

Donor ID	Pathology and genetics	Gender	Age	Lobe	Replicate	[Al] μg/g dry wt.
				T	1	0.24
					2	*2.54*
					3	1.05
				P	1	0.89
					2	1.87
					3	1.23
					Mean(SD)	4.33(7.23)
A7	Braak/BNE stage VI,cotton wool plaques (PSEN mutation) and limbic Lewy bodies dementia diagnosed at age 46	F	69	O	1	1.22
					2	1.62
					3	0.97
				F	1	1.08
					2	1.11
					3	**3.00**
				T	1	*2.69*
					2	1.19
					3	1.56
				P	1	0.54
					2	0.01
					3	0.32
					Mean(SD)	1.28(0.87)
A8	Braak stage VI with Lewy bodies	F	65	O	1	*2.44*
					2	1.73
	PSEN-1 (E280G)				3	*2.12*
				F	1	**5.06**
					2	*2.53*
					3	**35.65**
				T	1	**10.43**
					2	**6.31**
					3	**6.77**
				P	1	**3.82**
					2	0.36
					3	1.38
					Mean(SD)	6.55(9.59)
A9	Braak Stage V–VI, Lewy bodies	F	72	O	1	*2.53*
					2	*2.93*
	APP717(VAL-ILE)				3	0.81
				F	1	**8.56**
					2	1.28
					3	1.46
				T	1	*2.48*
					2	0.65
					3	*2.61*
				P	1	1.36
					2	**5.44**
					3	0.96
					Mean(SD)	2.59(2.30)
A10	Braak Stage VI, Lewy bodies	F	49	O	1	*2.38*
					2	**4.14**
	PSEN-1 (L153V)				3	*2.40*
				F	1	*2.60*
					2	0.63
					3	1.26
				T	1	*2.37*
					2	0.12
					3	1.99
				P	1	1.14
					2	0.76
					3	0.28
					Mean(SD)	1.67(1.18)
A11	Braak Stage V-VI	F	69	O	1	**23.93**
					2	0.85
	APP717(VAL-ILE)				3	**3.15**
				F	1	1.45
					2	0.84
					3	1.02
				T	1	1.41
					2	0.41
					3	**6.21**
				P	1	0.55
					2	*2.27*
					3	0.37
					Mean(SD)	3.54 (6.63)
A12	Braak at least Stage IV	M	61	O	1	**4.57**
					2	1.93
	APP717(VAL-GLY)				3	0.85
				F	1	1.81
					2	*2.54*
					3	0.84
				T	1	**6.00**
					2	0.27
					3	*2.47*
				P	1	**5.41**
					2	0.66
					3	0.01
					Mean(SD)	2.28(2.03)

aluminium content across all 12 individuals for each lobe were 3.89(5.86), 3.66(6.18), 2.03(2.35) and 1.61(1.90) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. The aluminium content of the parietal lobe was statistically different to the occipital lobe ($P = 0.029$; $df = 70$) but there were no other statistically significant differences in lobe aluminium content.

3.2. Aluminium fluorescence in brain tissues

Tissue sections from 6 of the 12 familial AD cases (A1, A5, A7, A8, A11 & A12) were investigated and the presence of aluminium was confirmed in each case using lumogallion and fluorescence microscopy. Representative images of positive aluminium fluorescence are shown in Figs. 1–3. Multiple deposits of aluminium were identified in each case and including in the brain of donor A1 which had the lowest mean aluminium content by TH GFAAS. Aluminium was primarily identified in grey matter as clusters or swathes of focal deposits some of which were densely stained and 1–5 μm in size while others were diffuse and up to 50 μm in length or diameter. Many of the former gave the impression of being intracellular being associated with astrocytes or neurons or tissue spaces vacated by dead or dying cells. The more diffuse deposits appeared to be extracellular and were similar in appearance to diffuse deposits of Aβ. Aggregates of lipofuscin were common in most tissues and some deposits of aluminium shared the appearance of accumulations of lipofuscin.

4. Discussion

The aluminium content of brain tissue donated by individuals with a diagnosis of familial and probable familial Alzheimer's disease was, overall, extremely high. The mean aluminium data for each lobe across all 12 donors are significantly higher ($P < 0.05$ in each case) than equivalent data (using identical methods and quality assurance indices) for the same lobes from a previous study which included 60 human brains of which *ca* 60% had been diagnosed as sporadic Alzheimer's disease [8,12]. Herein we measured some of the highest values recorded for individual samples of human brain tissue, for example to highlight just a few, 11.54 μg/g in the occipital lobe of donor A2, 13.41 μg/g in the frontal lobe of A5, 25.80 μg/g in the occipital lobe of A6, 35.65 μg/g in the frontal lobe of A8 and 23.93 μg/g in the occipital lobe of A11 (Table 1). These individual values are higher than previous measurements for non-AD and sporadic or late-onset AD brain tissue [7,8,12]. While we have previously recorded values up to *ca* 13.00 μg/g in AD with occupational exposure to aluminium [14] and one value of 23.00 μg/g in congophilic amyloid angiopathy (CAA) with environmental exposure to aluminium [13] the values measured herein for familial AD are more similar to those which have been associated with aluminium-induced encephalopathies [17]. It was of note that while the aluminium content of brain tissue from donor A1 was consistently low across all 4 lobes (mean (SD) 0.34(0.26) μg/g) when additional tissues from this donor were investigated by fluorescence microscopy significant deposits of aluminium were identified across all 4 lobes. This observation tends to support the previous contention of focal as opposed to homogeneous distribution of aluminium in human brain tissues [7,14].

The presence of aluminium in brain tissue from donors with familial AD was fully supported qualitatively by aluminium-specific fluorescence microscopy (Figs. 1–3). We have produced striking and unequivocal images of aluminium in human brain tissue and confirmed aluminium's presence in each of the familial AD brains investigated. Aluminium appeared to be both intracellular, associated with dead and dying neurones and extracellular in large diffuse deposits. Further detailed research will be required to confirm the precise locations of deposits of aluminium. Our images may support previous studies using various semi-quantitative techniques which suggested the co-localisation of aluminium with; neurones and neurofibrillary tangles in AD [10] and dementia pugilistica [18]; Lewy bodies in Parkinson's disease [19]; lipofuscin in AD [20] and senile plaques in AD [21]. We have previously identified the co-localisation of aluminium and amyloid in an AD brain [7] and we have additional evidence herein to support this (Fig. 4). Aluminium is shown in the very near vicinity of a plaque-like structure which stained positively with Congo red and gave apple-green birefringence under polarised light. However, emission spectra for Congo red and lumogallion show significant overlap and so additional research will be required to provide unequivocal evidence by fluorescence microscopy for the association of aluminium with amyloid in diseased human brain tissue.

These data, supported by visual evidence of aluminium in brain tissue, raise the possibility that genetic predisposition to AD is accompanied by a higher propensity to accumulate and retain aluminium in the brain. In familial AD the expression and metabolism of APP is altered and there is substantial evidence of a role for Aβ in disease aetiology [6]. The observation that Aβ binds aluminium promoting its precipitation in a β-sheet conformation [22,23] alongside the known co-localisation of aluminium and Aβ in human brain tissue [7] might suggest a role for Aβ in the retention and accumulation of aluminium in brain tissue in familial AD.

5. Conclusions

Aluminium is neurotoxic [11,23] and the concentrations of aluminium found in these familial AD brains are unlikely to be benign and indeed are highly likely to have contributed to both the onset and the aggressive nature of any ongoing AD in these individuals. These data lend support to the recent conclusion that brain aluminium will contribute towards all forms of AD under certain conditions [15].

Competing interests

The authors declare that they have no competing interests.

Author contributions

AK and CT organised tissue donations, confirmed diagnoses of fAD and performed neuropathological examinations. AM and CE measured Al content of tissue and AM performed all light and fluorescence microscopy. CE wrote the manuscript. All authors read and commented on the final manuscript.

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