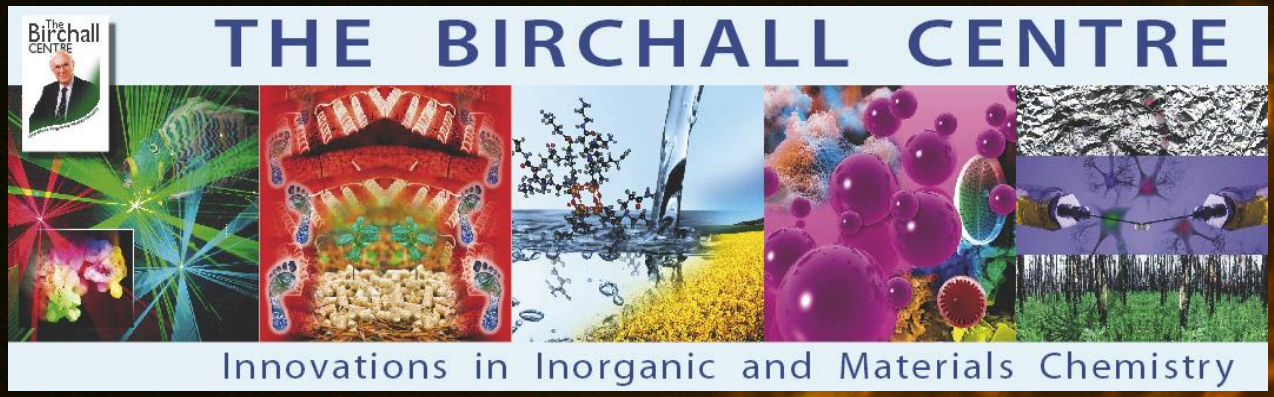

Visualising aluminium in human brain tissue in autism and multiple sclerosis

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Keele UNIVERSITY

Autism spectrum disorder (ASD)

- Genetic and environmental factors are thought to be associated with the onset and progression of ASD.
- Human exposure to aluminium has been implicated in ASD.
- Animal models of ASD support a connection with the use of aluminium adjuvants in human vaccination.
- First study to assess quantitatively (TH-GFAAS) and qualitatively (lumogallion fluorescence) the presence of aluminium in brain tissue from donors who died with a diagnosis of ASD.

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Aluminium in brain tissue in autism

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ABSTRACT

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.

Donor	Sex	Age	Lobe	Mean [Al] µg/g (SD)	Highest [Al] µg/g
A1	**F**	**44**	O	1.69 (2.22)	4.26
			F	1.01 (0.08)	1.10
			T	1.14 (0.02)	1.16
			P	0.86 (0.45)**	1.18
			All	**1.20 (1.06)**	-
A2	**M**	**50**	O	5.03 (2.46)	7.87
			F	1.13 (0.45)	1.65
			T	1.69 (0.92)	2.73
			P	6.41 (10.54)	18.57
			H	1.42*	1.42
			All	**3.40 (5.00)**	-
A3	**M**	**22**	O	1.10 (0.79)	2.01
			F	2.86 (1.22)	4.14
			T	2.81 (1.33)	4.25
			P	2.82 (1.81)	5.18
			All	**2.40 (1.58)**	-

Aluminium content measured by TH-GFAAS of occipital (O), frontal (F), temporal (T) and parietal (P) lobes and hippocampus (H) of brain tissue from donors with **autism spectrum disorder** (ASD) (*n* = 3 unless indicated ***n* = 2, or **n* = 1).

- *Pathologically concerning:*

[Al] ≥ 2.00 µg/g dry wt.

- *Pathologically significant:*

[Al] ≥ 3.00 µg/g dry wt.

Brain aluminium content in ASD *contd*.

Donor	Sex	Age	Lobe	Mean [Al] µg/g (SD)	Highest [Al] µg/g
A4	**M**	**15**	O	8.74 (11.59)	22.11
			F	2.00 (1.10)	3.23
			T	1.49 (0.37)	1.83
			P	4.05 (3.77)**	6.71
			H	0.02*	0.02
			All	**3.73 (6.02)**	-
A5	**M**	**33**	O	2.54 (0.74)	3.13
			F	5.62 (3.75)**	8.27
			T	6.82 (8.91)	17.10
			P	4.21 (1.87)**	5.53
			All	**4.77 (4.79)**	-

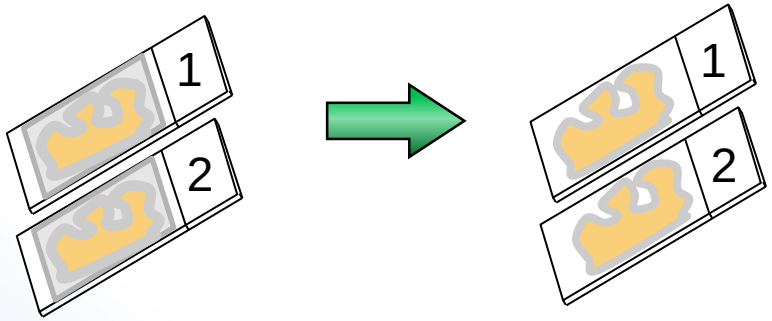
Aluminium content measured by TH-GFAAS of occipital (O), frontal (F), temporal (T) and parietal (P) lobes and hippocampus (H) of brain tissue from donors with **ASD** (*n* = 3 unless indicated ***n* = 2, or **n* = 1).

(Mold *et al*., 2018)

Pathologically concerning: [Al] ≥2.00 µg/g dry wt.
Pathologically significant: [Al] ≥3.00 µg/g dry wt.

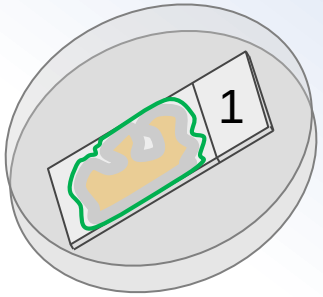
Tissue preparation and staining

① De-waxing



Sections de-waxed in Histoclear™ and gradually rehydrated from 100% ethanol to ultrapure water.

② PAP pen



Hydrophobic barrier created using a PAP pen.

③ Staining

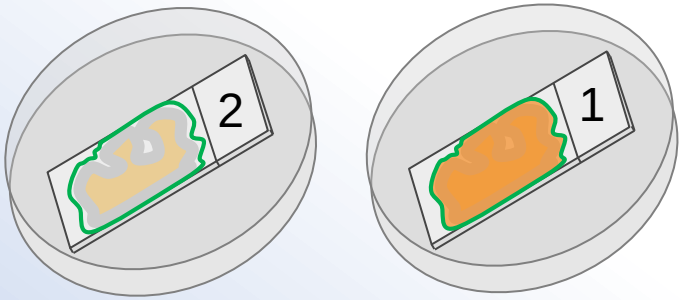
Sections stained for 45 minutes with:

1. 1mM lumogallion in 50mM PIPES buffer pH 7.4.

2. PIPES buffer only (autofluorescence).

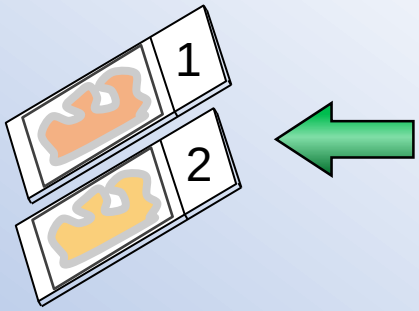
Lumogallion

Autofluorescence



PIPES / H_2O rinses

④ Mounting



Fluormount™, 24h

Patient ID: A2
Lobe: Hippocampus
Age: **50**
Sex: **M**

Aluminium in the vasculature

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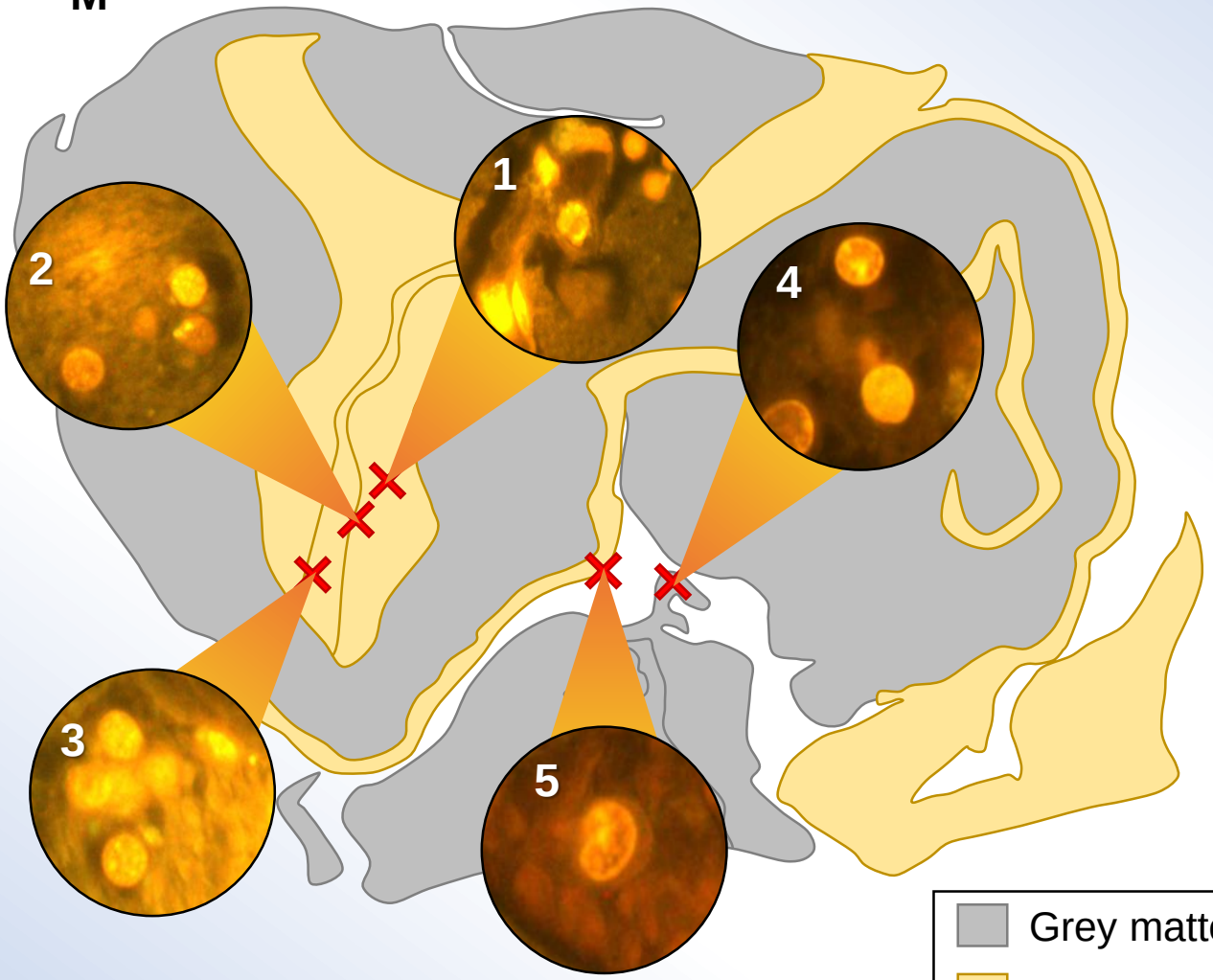
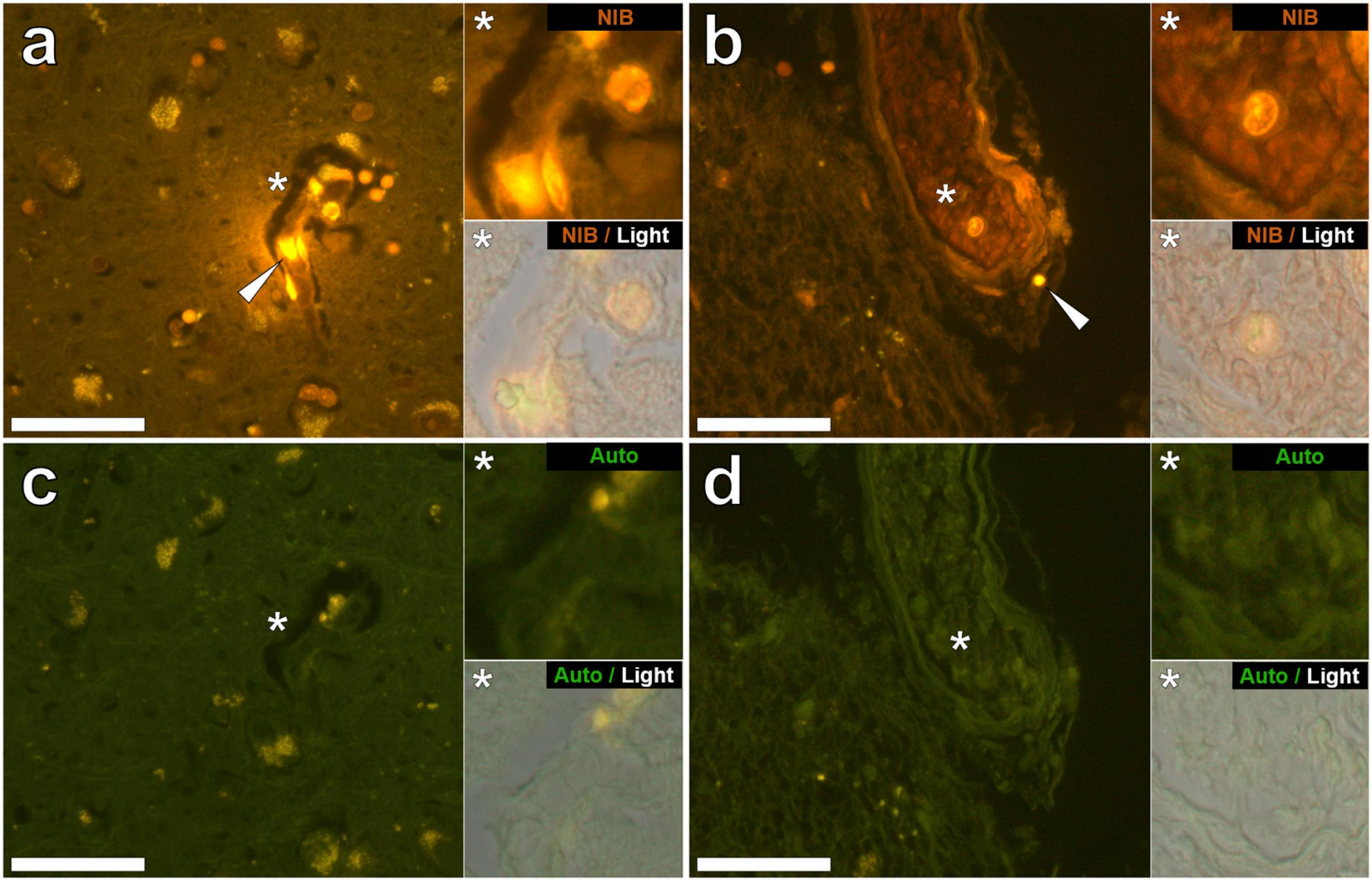


Oxford Brain Bank (OBB) ID: TW404

- **A2: Hippocampus, 50-year-old Male**



Intracellular lumogallion-reactive aluminium in the **vasculature** (**a – d**) of the hippocampus of a 50-year-old male donor with autism.

Patient ID: A2
Lobe: Frontal
Age: **50**
Sex: **M**

Aluminium in leptomeningeal membranes

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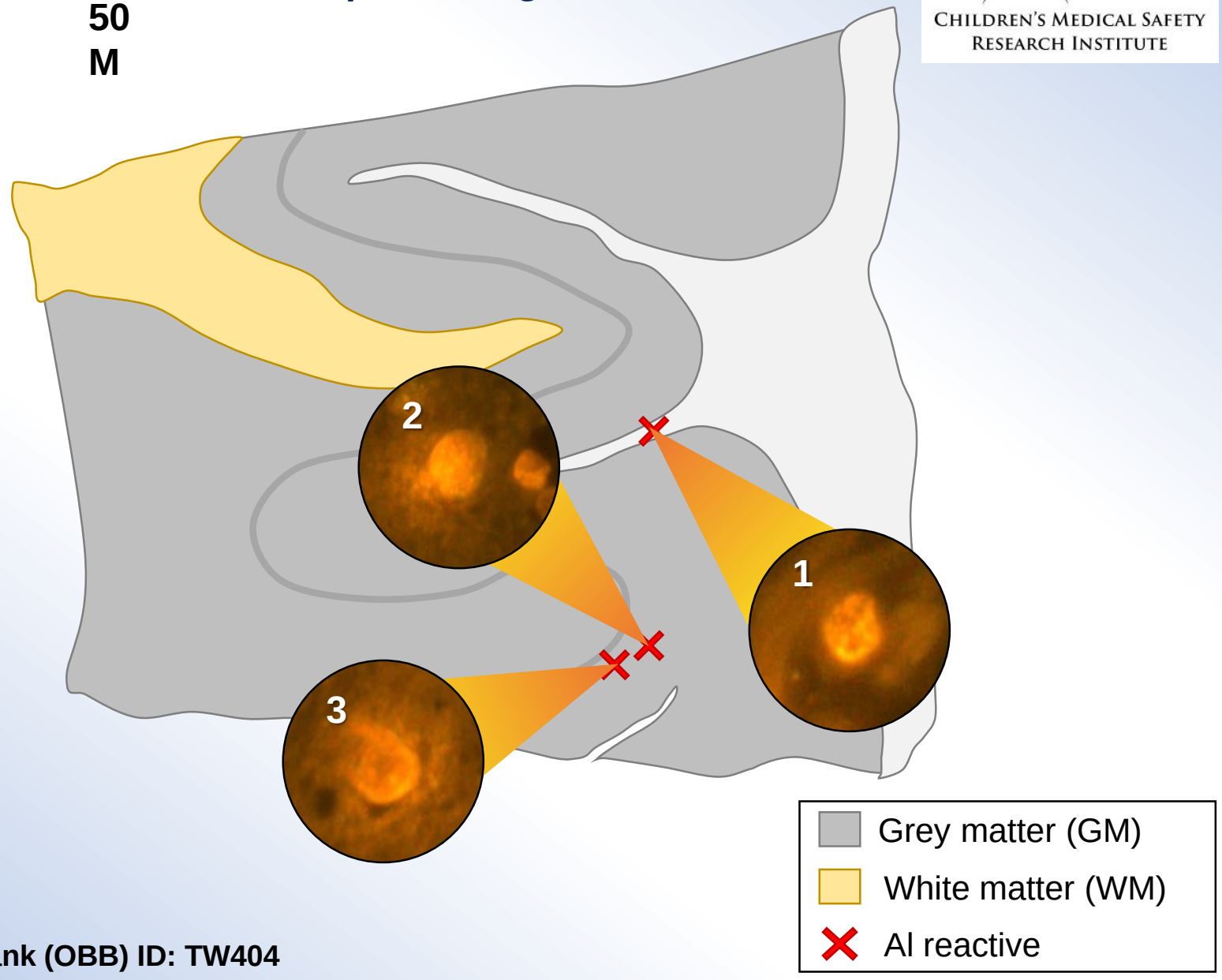
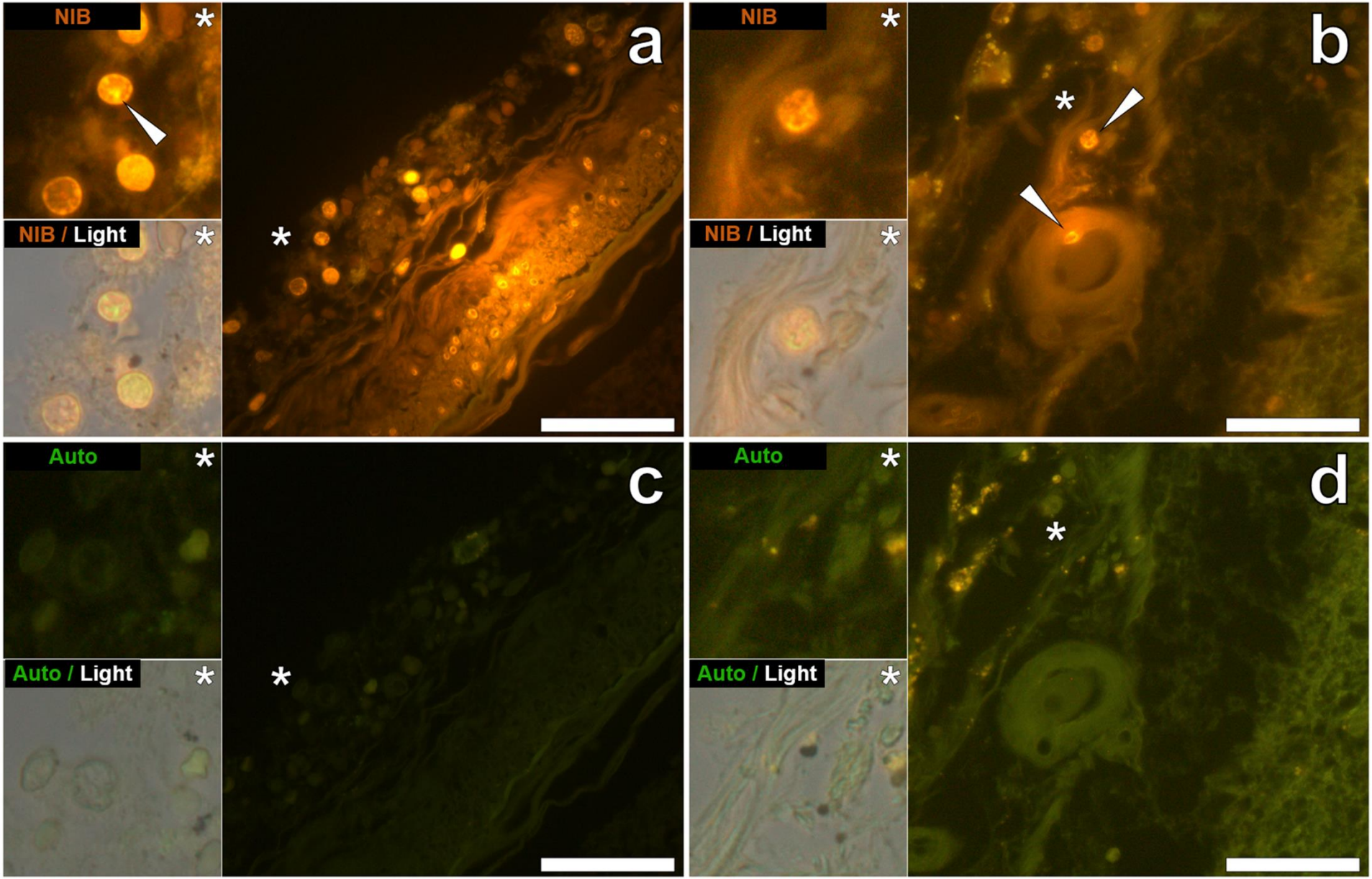


Oxford Brain Bank (OBB) ID: TW404

- **A2: Hippocampus & frontal lobe, 50-year-old Male**



Mononuclear inflammatory cells (lymphocytes) in **leptomeningeal** membranes in the hippocampus (**a & c**) and frontal lobe (**b & d**) of a 50-year-old male donor with autism.

Patient ID: A4
Lobe: Hippocampus
Age: **15**
Sex: **M**

Aluminium in a young donor with ASD

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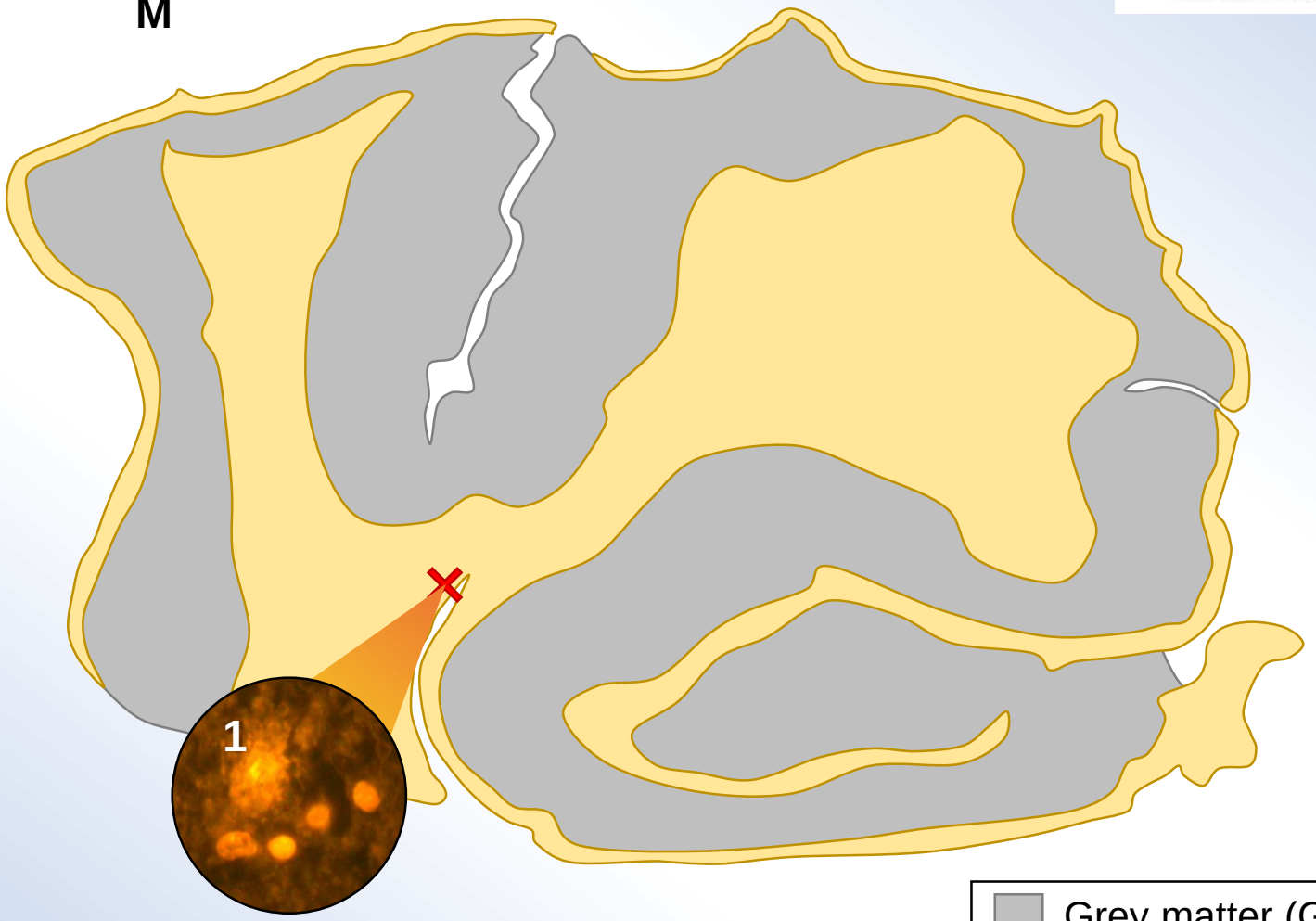

Oxford Brain Bank (OBB) ID: TW404

Patient ID: A4
Lobe: Parietal
Age: **15**
Sex: **M**

Aluminium in a young donor with ASD

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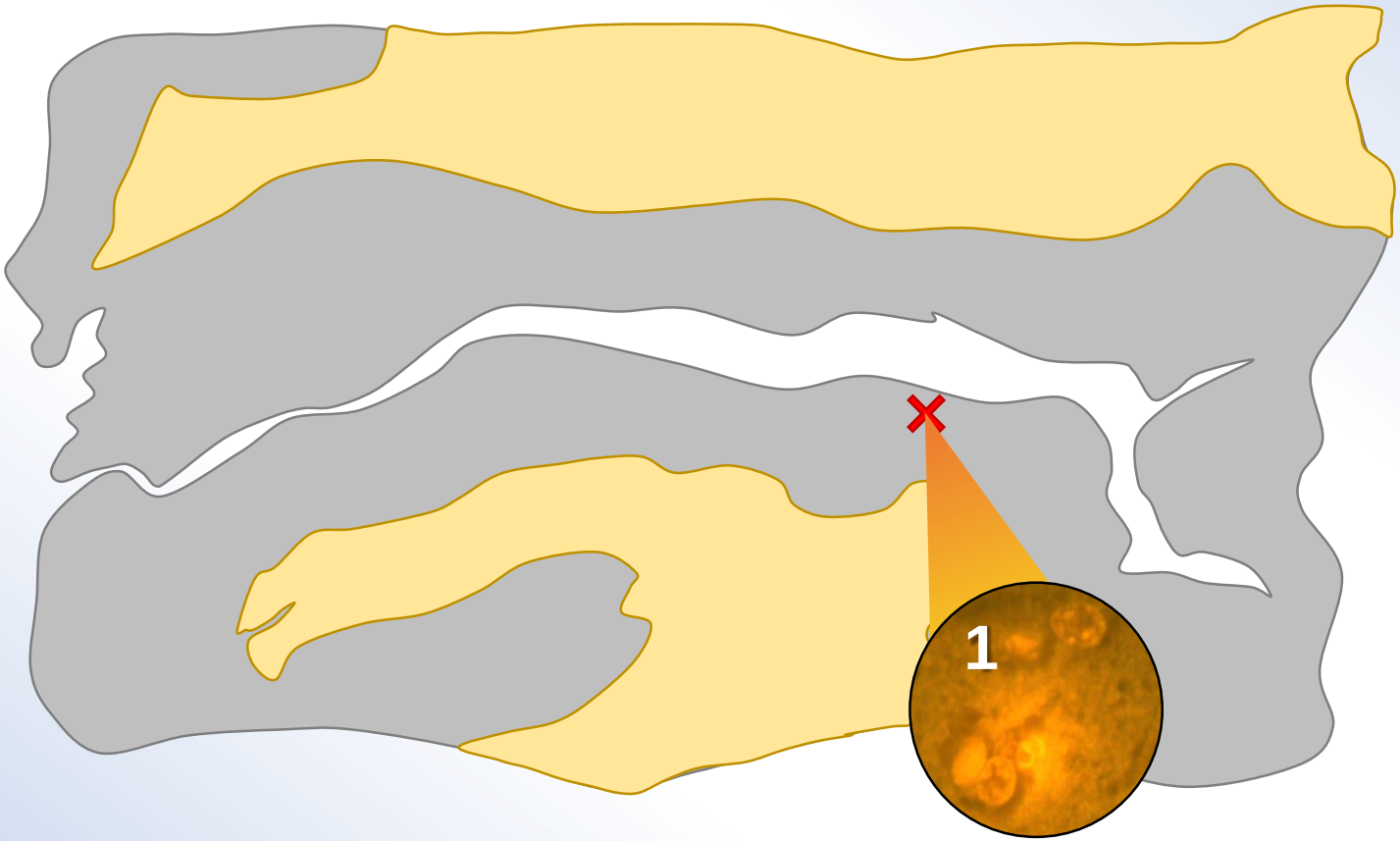
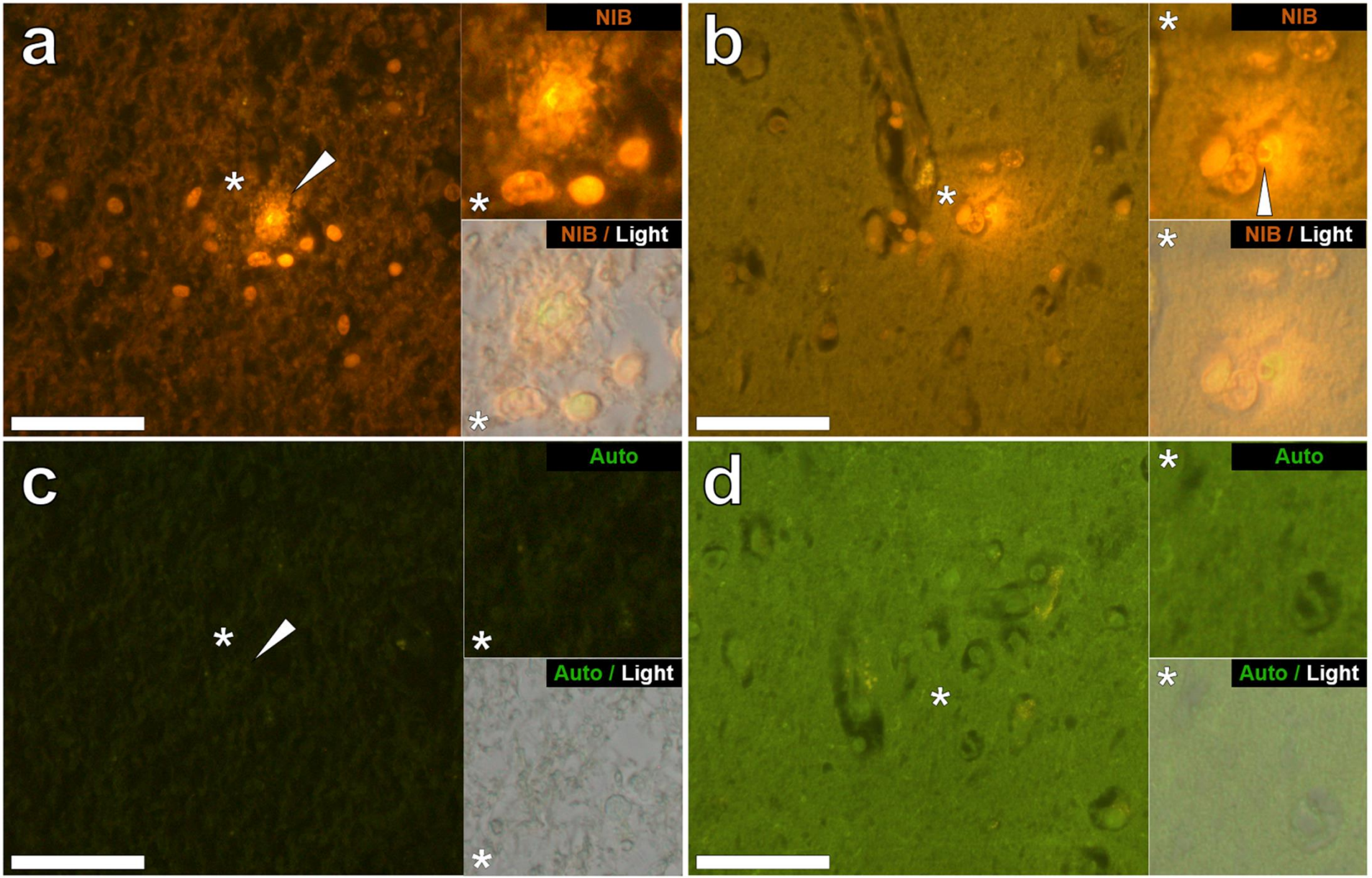


Oxford Brain Bank (OBB) ID: TW404

- **A4: Hippocampus & parietal lobe, 15-year-old Male**



Intracellular aluminium in **glia** in the hippocampus (**a & c**) and a **neuronal** cell in the parietal lobe (**b & d**) of a 15-year-old male donor, diagnosed with autism.

Patient ID: A4
Lobe: Temporal
Age: **15**
Sex: **M**

Aluminium in microglial cells

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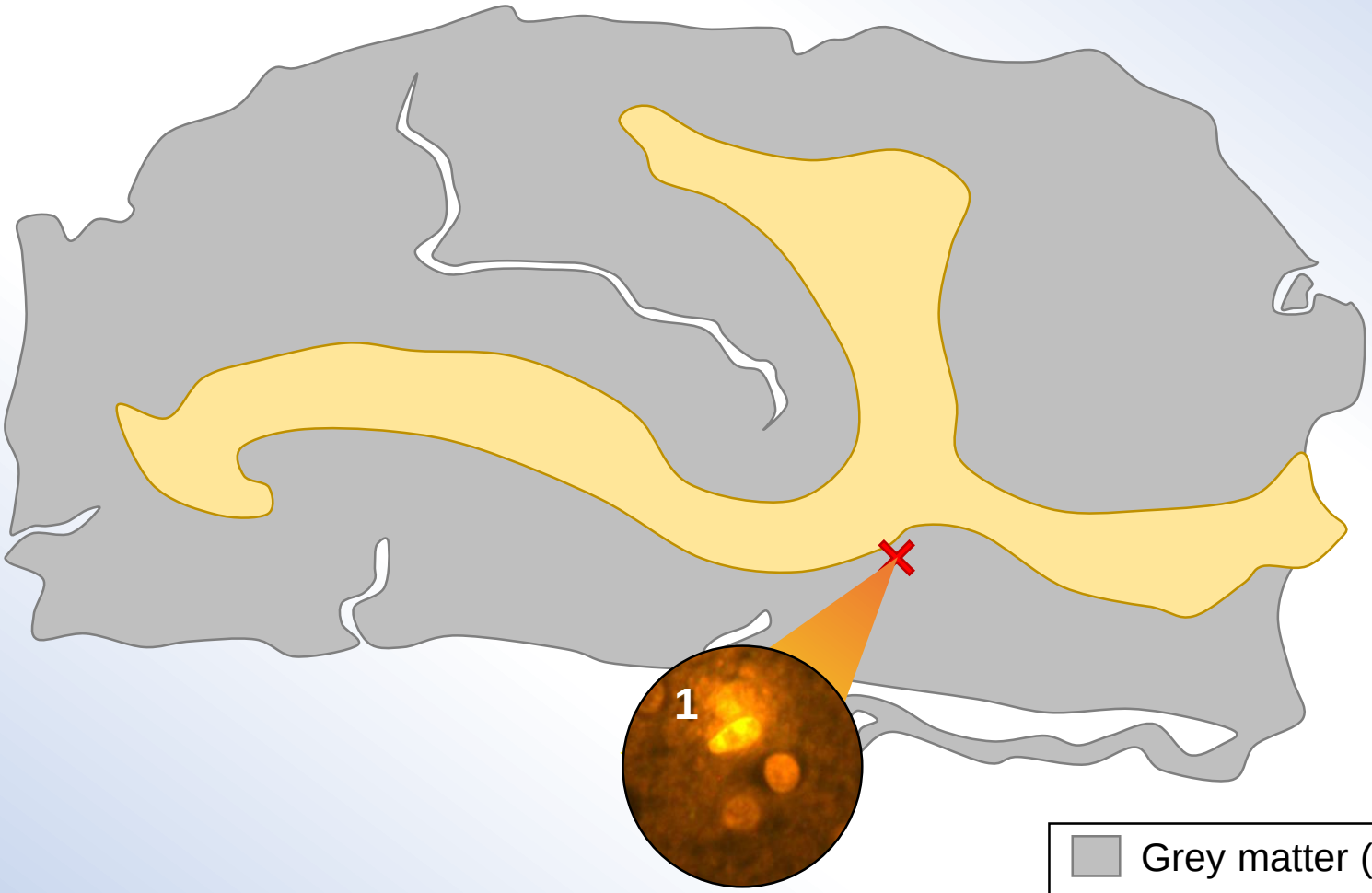

Oxford Brain Bank (OBB) ID: TW404

Patient ID: A8
Lobe: Parietal
Age: **29**
Sex: **M**

Aluminium in microglial cells

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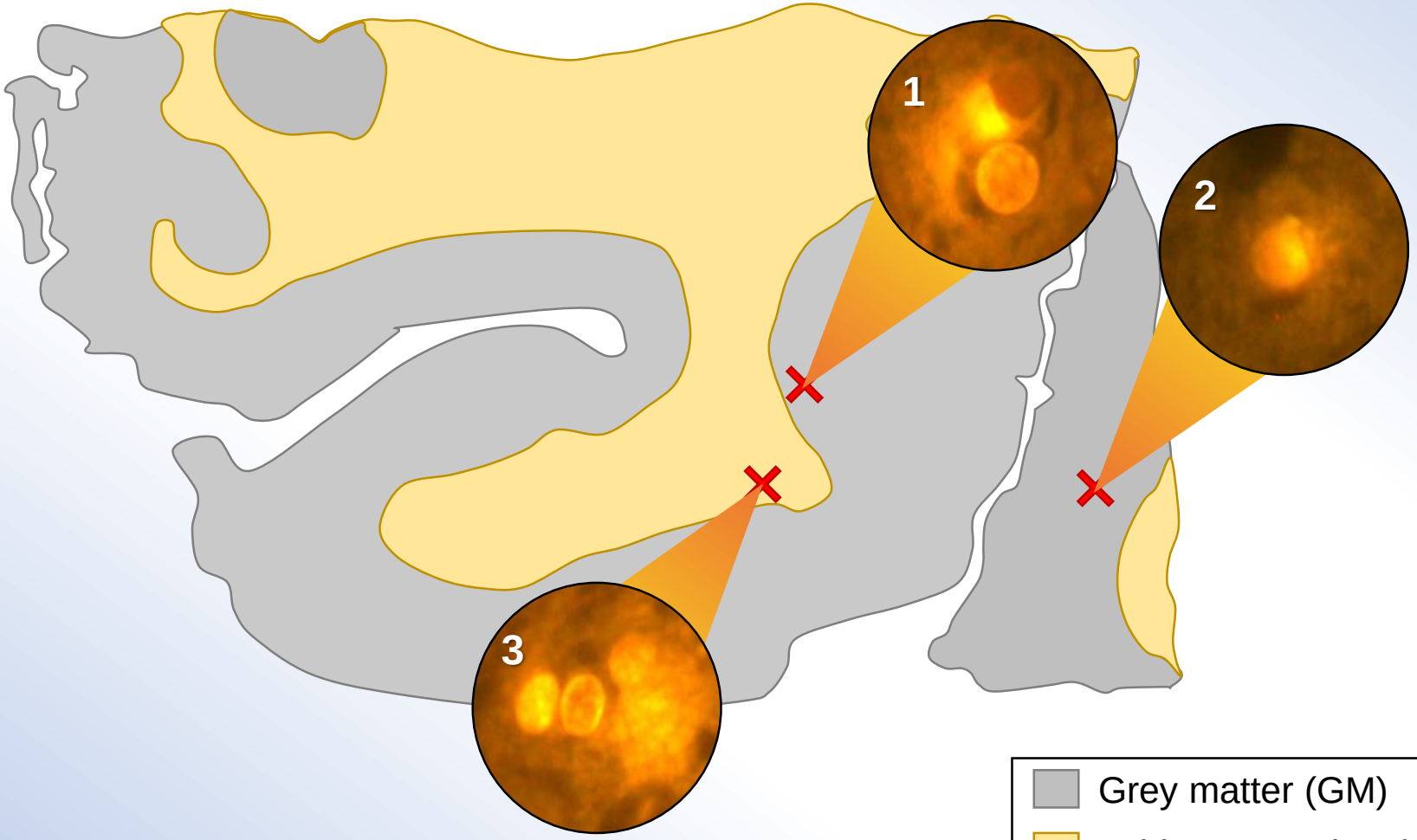
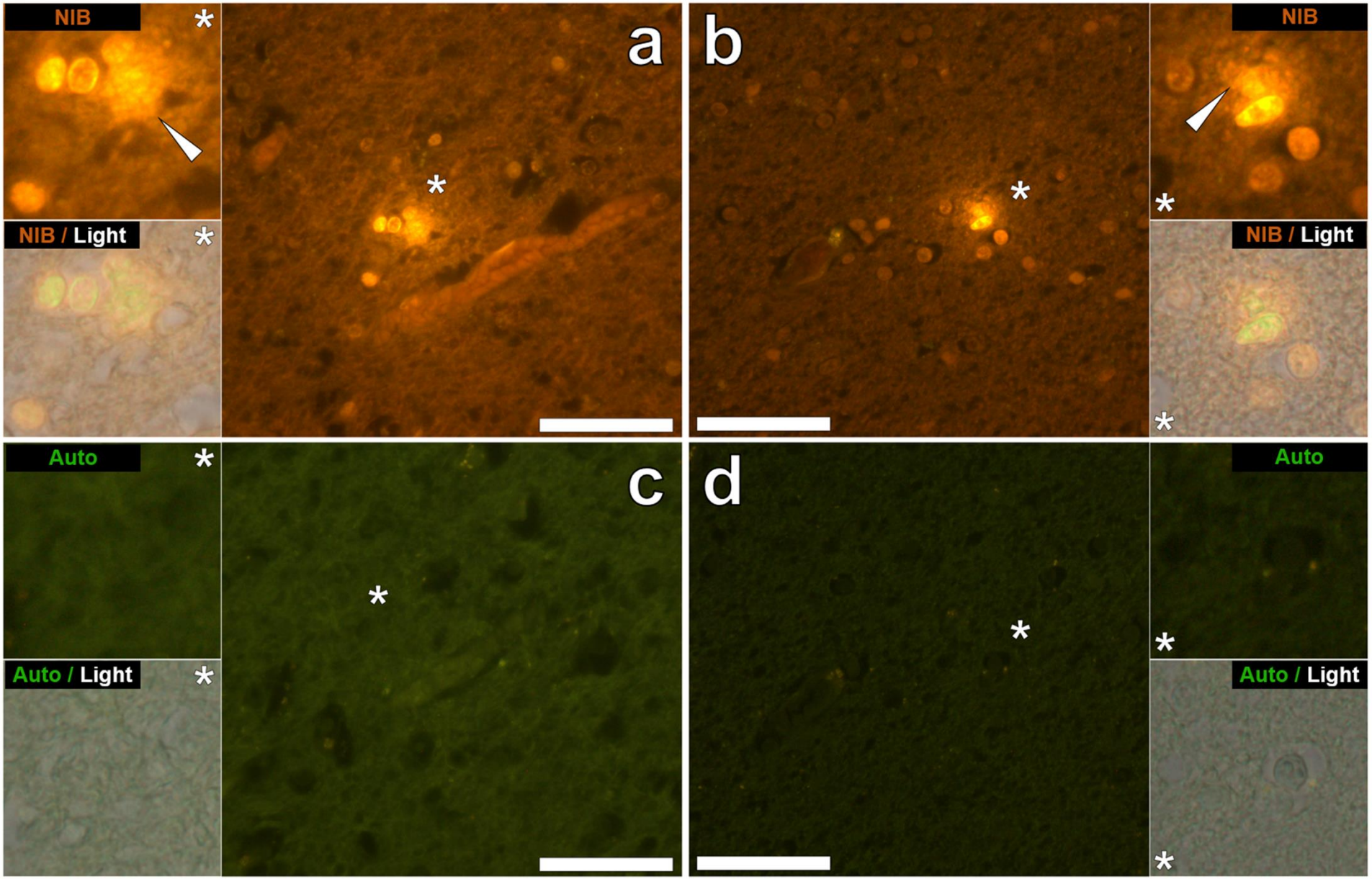


Oxford Brain Bank (OBB) ID: TW404

- **A8: Parietal lobe, 29-year-old Male & A4: Temporal lobe, 15-year-old Male**



Intracellular aluminium in cells compatible with **microglia** in the parietal (**a & c**) and temporal (**b & d**) lobes of 29 and 15-year-old male donors, diagnosed with autism.

Patient ID: A10
Lobe: Temporal
Age: **14**
Sex: **M**

Aluminium in a second young donor with ASD

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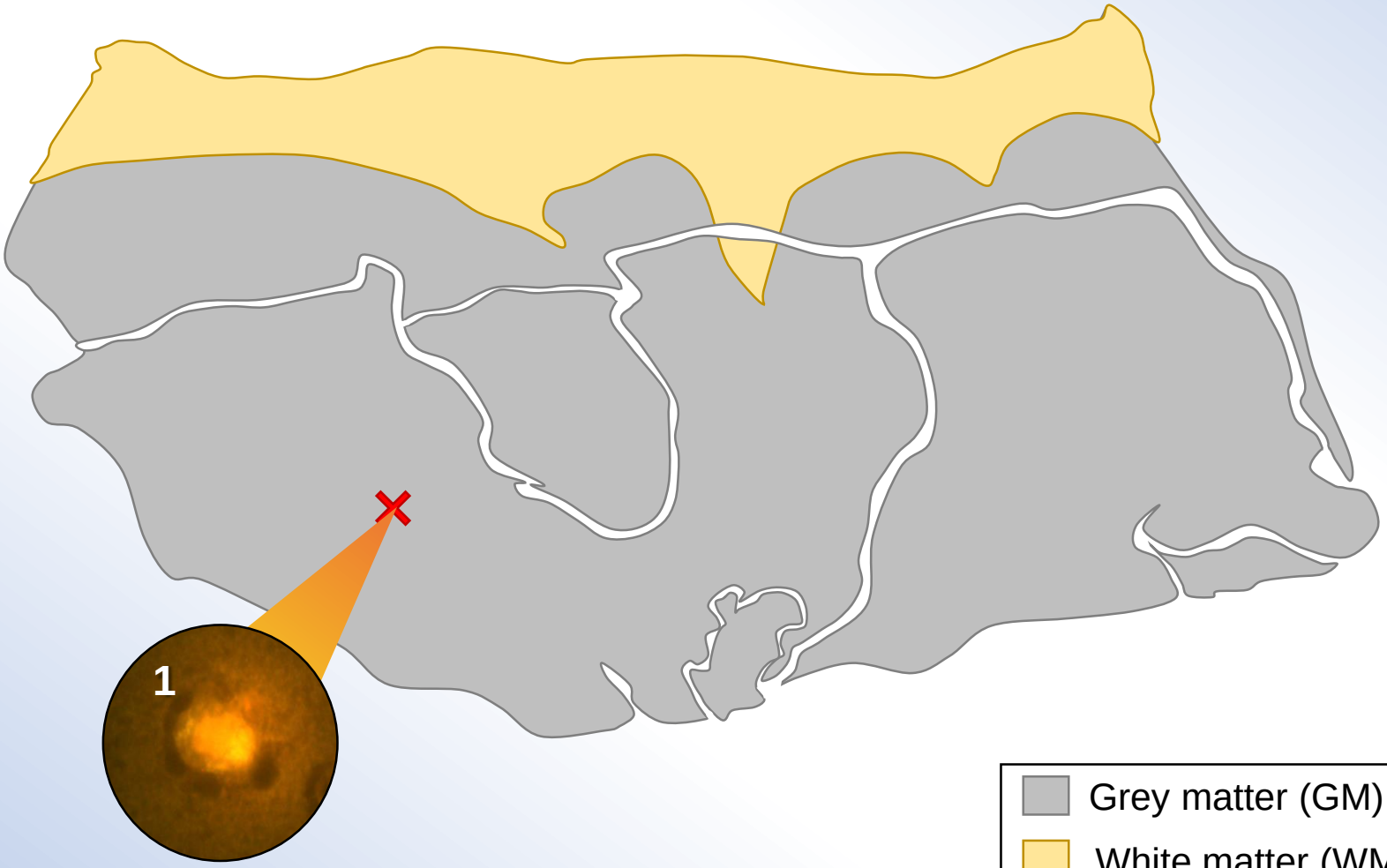

Oxford Brain Bank (OBB) ID: TW404

Patient ID: A10
Lobe: Hippocampus
Age: **14**
Sex: **M**

Aluminium in a second young donor with ASD

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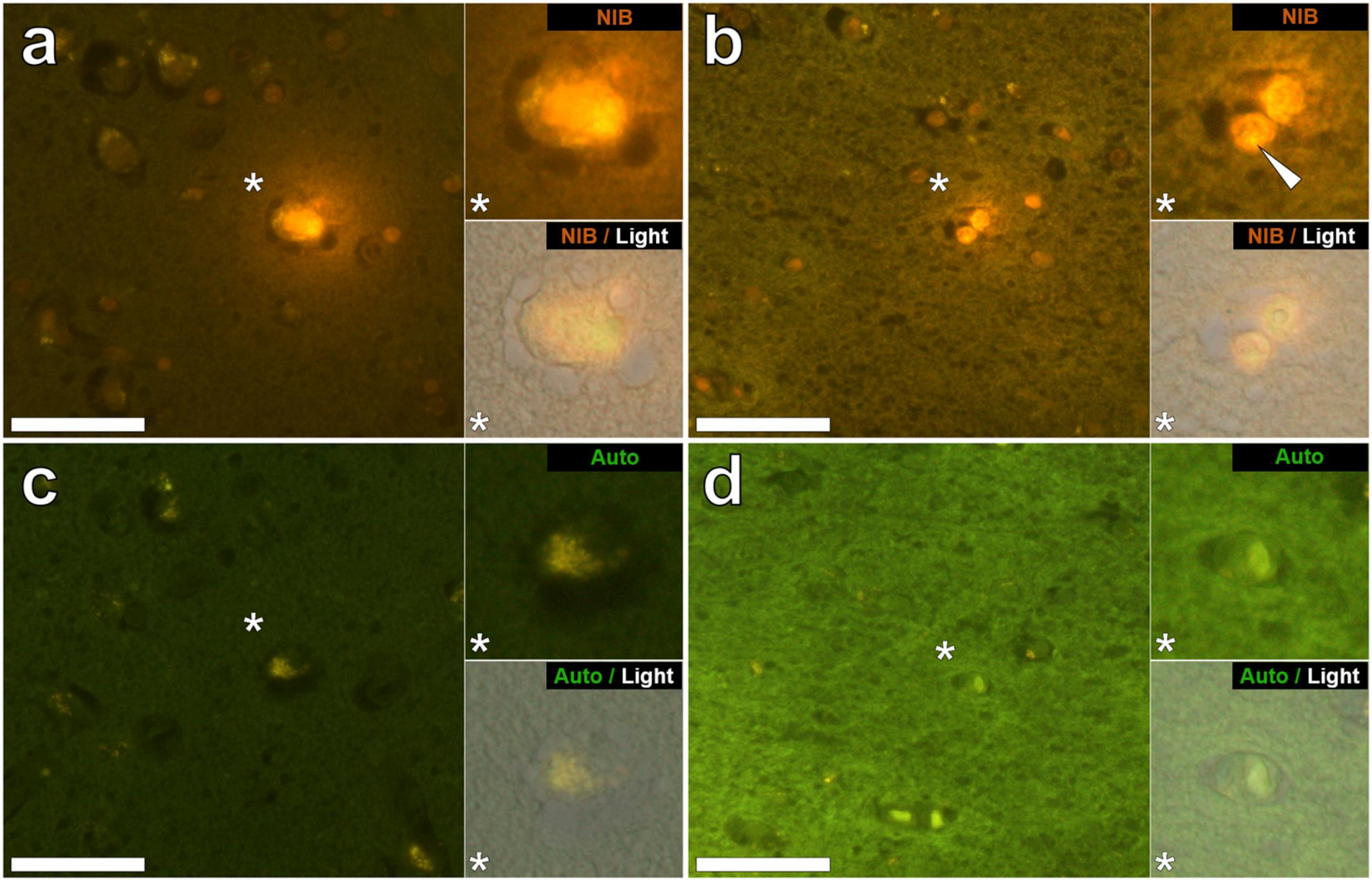
Oxford Brain Bank (OBB) ID: TW404

- **A10: Temporal lobe & hippocampus, 14-year-old Male**



Intracellular aluminium in **neuronal** and **glial** cells in the temporal lobe (**a & c**) and hippocampus (**b & d**) of a 14-year-old male donor, diagnosed with autism.

Multiple sclerosis (MS)

- Chronic, immune-mediated, demyelinating disease of the central nervous system of unknown aetiology, though more understood than ASD.
- Genetic and environmental factors are thought to be associated with the onset and progression of MS, as with ASD.
- Human exposure to aluminium has been implicated in MS.
- Individuals with MS have been shown to excrete large amounts of aluminium in their urine **(Exley *et al*., 2013, Jones *et al*., 2017)**.
- First measurements and imaging of aluminium in human brain tissue from donors with MS (manuscript in preparation).

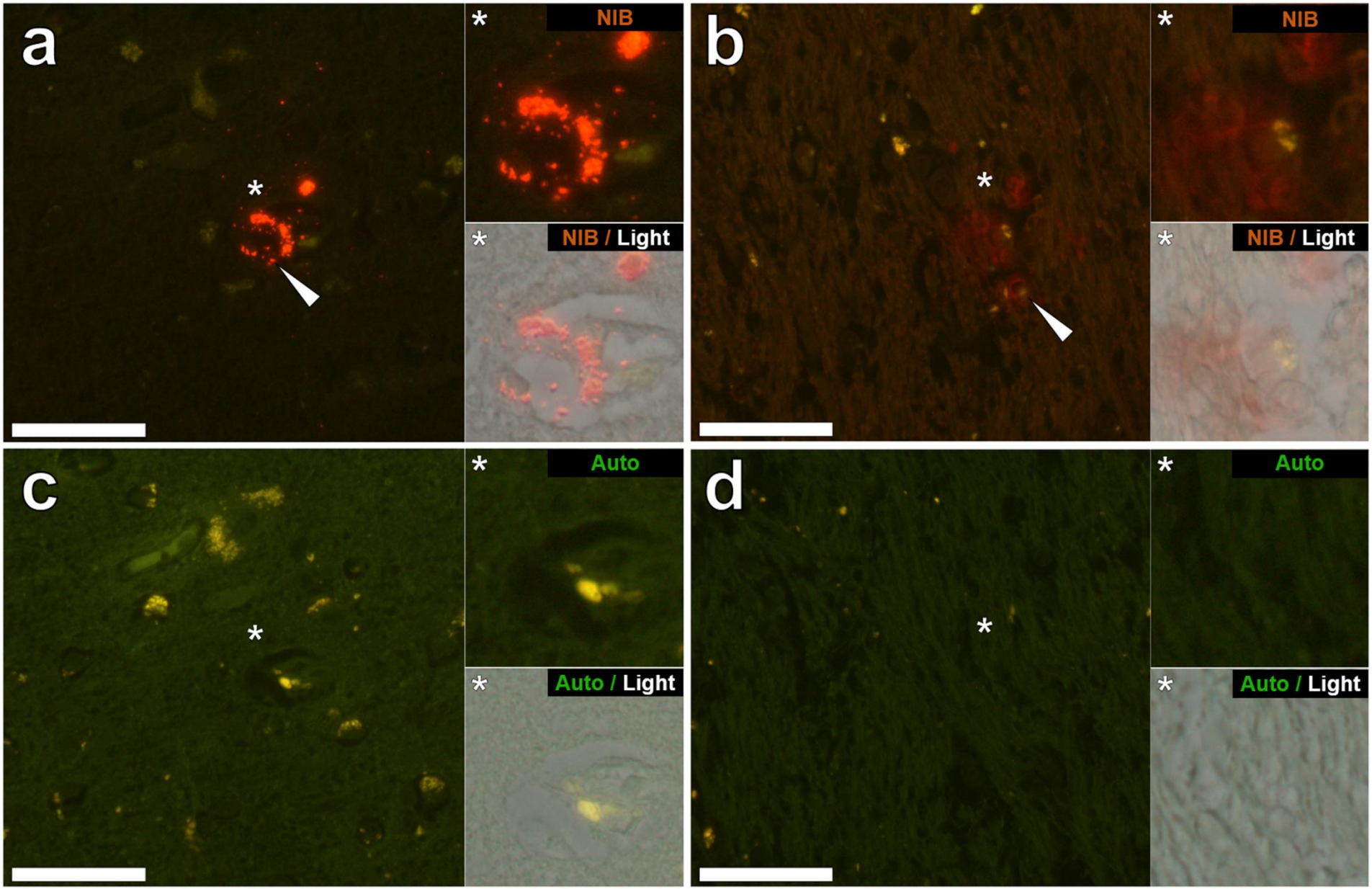
Brain aluminium content in MS

Aluminium content measured by TH-GFAAS of frontal, temporal, occipital and parietal lobes of brain tissue from donors with **MS**. RPMS - relapsing progressive MS, RRMS – relapsing remitting MS, SPMS – secondary progressive MS.

Donor	Sex	Age	MS	[Al] µg/g dry wt. mean (SD) [*n*]			
				Frontal	Temporal	Occipital	Parietal
MS107	M	38	RPMS	3.41 (3.54) [5]	0.59 (0.64) [3]	0.58 (0.04) [2]	9.84 (16.70) [17]
MS274	**M**	**56**	**RRMS**	29.14(57.92) [5]	3.53 (2.55) [13]	0.50 (0.57) [10]	0.36 (0.20) [3]
MS356	F	45	SPMS	1.84 (2.85) [5]	1.81 (1.78) [5]	1.40 (1.86) [16]	1.51 (2.44) [8]
MS401	F	82	SPMS	0.65 (0.65) [4]	1.55 (1.96) [16]	5.66 (9.27) [20]	2.36 (1.65) [8]
MS317	**F**	**48**	**SPMS**	5.44 (5.73) [5]	NA	NA	NA

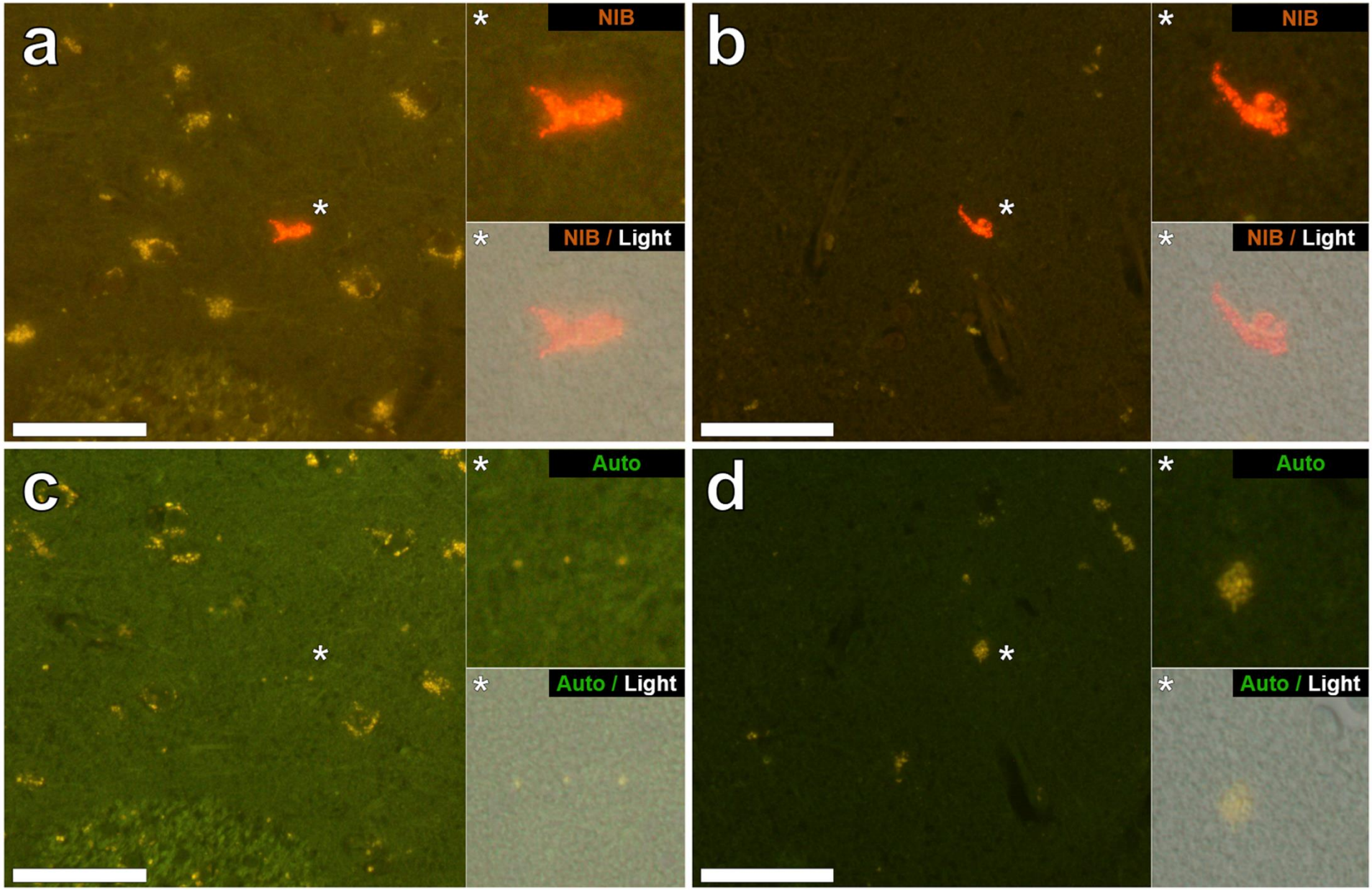
Pathologically concerning: ≥2.00 µg/g; Pathologically significant: ≥3.00 µg/g.

- **MS274: Frontal lobe & hippocampus, 56-year-old Male**



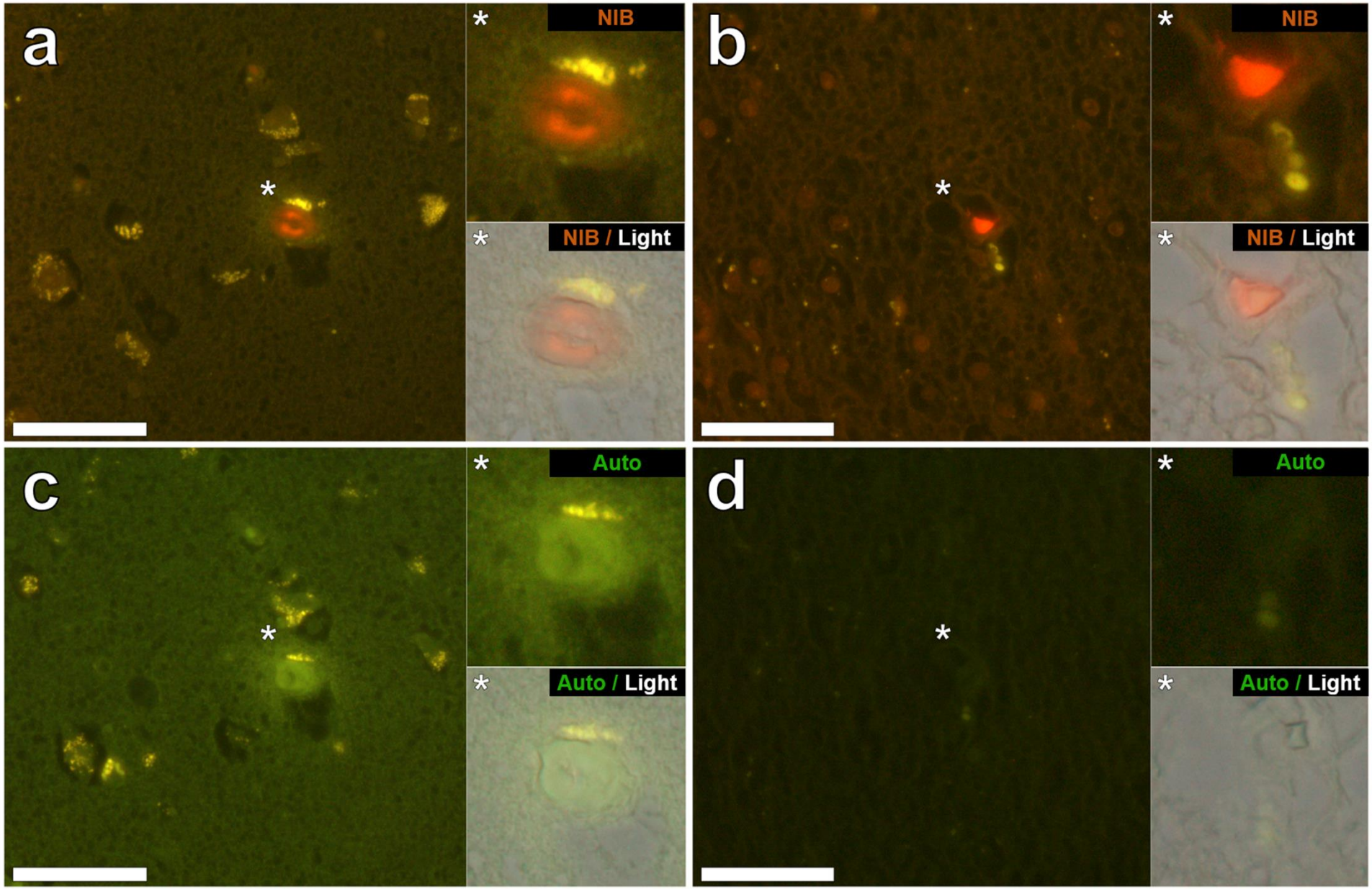
Punctate and diffuse **extracellular** aluminium in the frontal lobe (**a & c**) and hippocampus (**b & d**) of a 56-year-old male donor, diagnosed with RRMS.

- **MS274: Temporal lobe & hippocampus, 56-year-old Male**



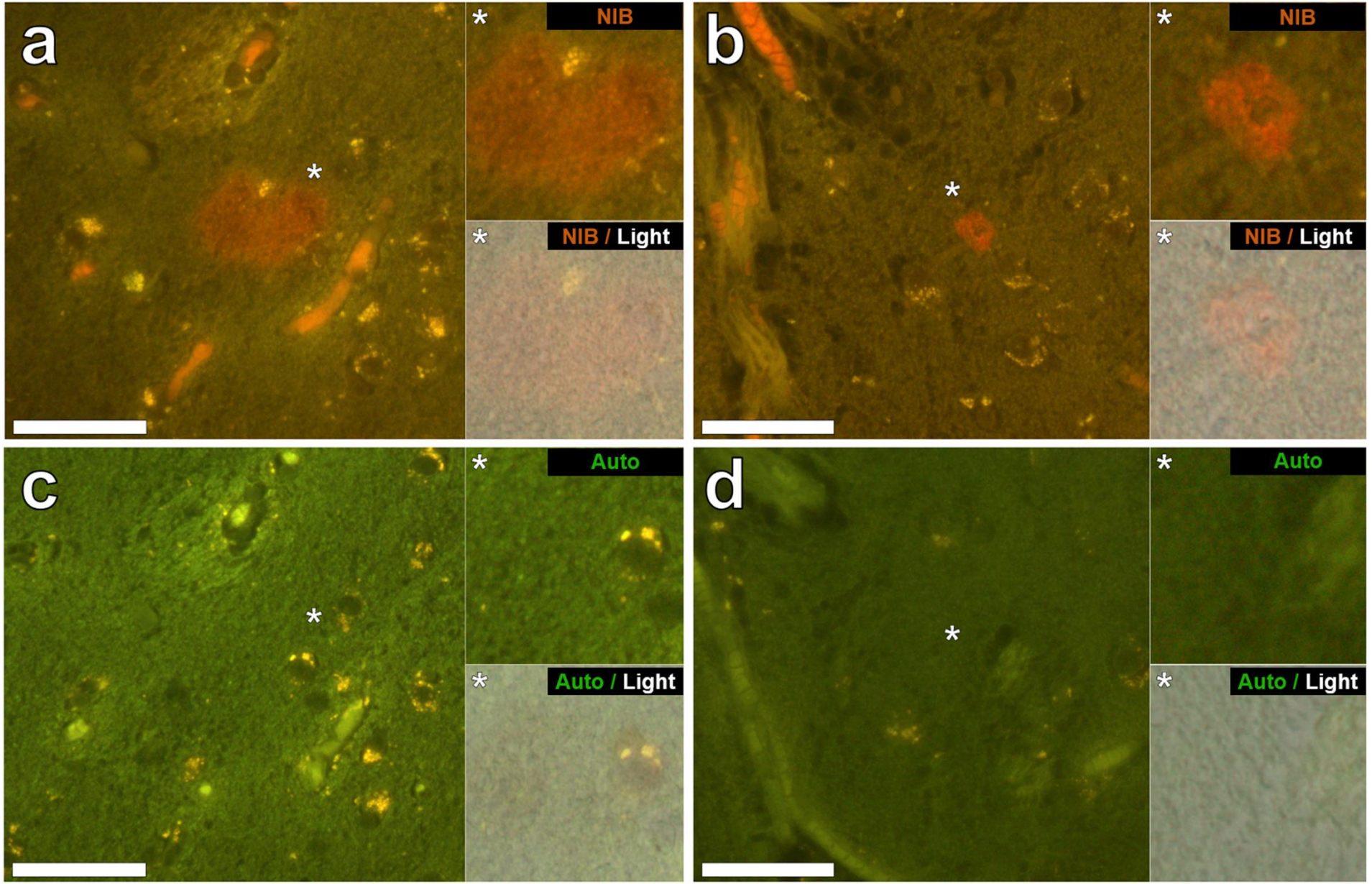
Intracellular aluminium in glial cells in the internal capsule (**a & c**) and hippocampus (**b & d**) of a 56-year-old male donor, diagnosed with RRMS.

- **MS317: Frontal lobe & hippocampus, 48-year-old Female**



Aluminium identified in ***corpora amylacea*** in the frontal lobe (**a & c**) and **glia** in the para-hippocampal gyrus (**b & d**) of a 48-year-old female donor, diagnosed with SPMS.

- **MS274 & MS317: Temporal lobes, 56-year-old Male & 48-year-old Female**



Diffuse extracellular aluminium in basal ganglia of the male donor (RRMS) (**a & c**) and medial temporal region of the female donor (SPMS) (**b & d**).

Conclusions

- Aluminium deposited in brain tissue in ASD was found to be intracellular and predominantly in microglial-like and other inflammatory non-neuronal cells.
- Aluminium content in ASD provided some of the highest measurements yet recorded in brain tissue with an exceptionally high amount noted for a 15-year-old boy of 8.74 (11.59) μg/g dry wt. (mean, SD).
- Aluminium deposited in brain tissue in MS was predominantly extracellular with intracellular aluminium primarily noted in microglial-like cells.
- Co-deposition of aluminium with *corpora amylacea* may suggest a role for the metal ion in neurodegeneration in MS.
- Aluminium content in MS was universally high with concentrations often exceeding 10 μg/g dry wt.

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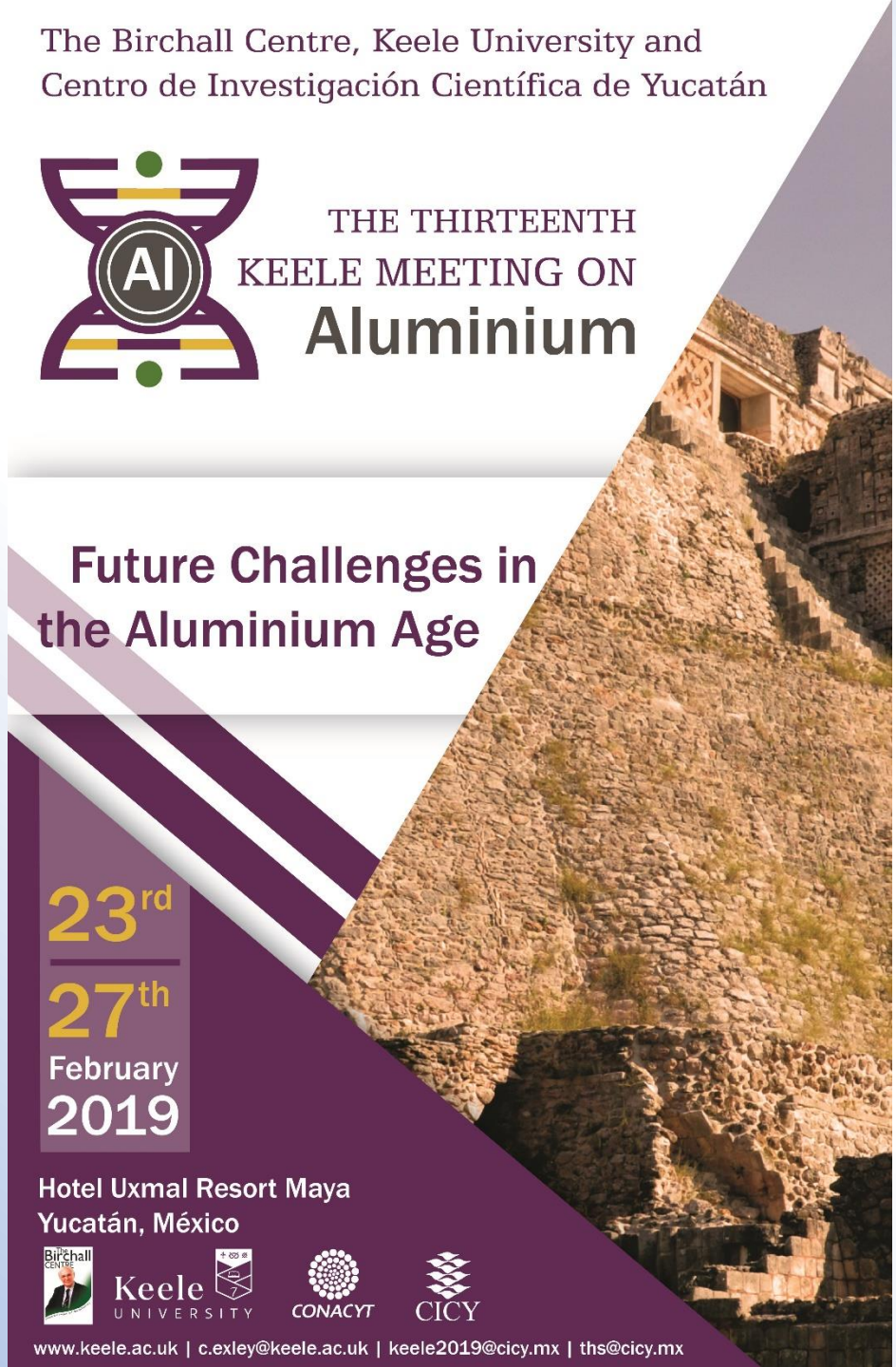

Time to get ready for KEELE13!

Come to the Thirteenth Keele Meeting on Aluminium in February 2019 in Mexico.

See our website https://www.keele.ac.uk/aluminium for details.

Acknowledgements

Funding:

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Bioinorganic chemistry of Al and Si research group:

- **Professor Christopher Exley**
- Dr Emma Shardlow
- Dr Ambreen Mirza
- Isabel Rodriguez (Ph.D. candidate)

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- **Dr Andrew King** (Kings College Hospital, London, UK).
- Oxford Brain Bank, Oxford, UK.
- MS Society Tissue Bank, London, UK.

University of Zaragoza:

- Professor Lluís Luján
- Javier Asin (Ph.D. candidate)