Epigenetic age acceleration is associated with oligodendrocyte proportions in MSA and control brain tissue

Megha Murthy[1,2], Gemma Shireby[3], Yasuo Miki[1,4], Emmanuelle Viré[5], Tammaryn Lashley[1,6], Thomas T. Warner[1,2,7], Jonathan Mill[3], Conceição Bettencourt[1,6*]

[1]Queen Square Brain Bank, UCL Queen Square Institute of Neurology, University College London, UK

[2]Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK.

[3]University of Exeter Medical School, University of Exeter, UK

[4]Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki, Japan.

[5]UCL Institute of Prion Diseases, MRC Prion Unit at UCL, University College London, London, UK.

[6]Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK.

[7]Reta Lila Weston Institute, UCL Queen Square Institute of Neurology, London, UK.

***Corresponding Author:**

Conceição Bettencourt, PhD

Department of Clinical and Movement Neurosciences

UCL Queen Square Institute of Neurology

Queen Square Brain Bank for Neurological Disorders

1 Wakefield Street

London WC1N 1PJ

United Kingdom

Email: c.bettencourt@ucl.ac.uk

Supplementary Information

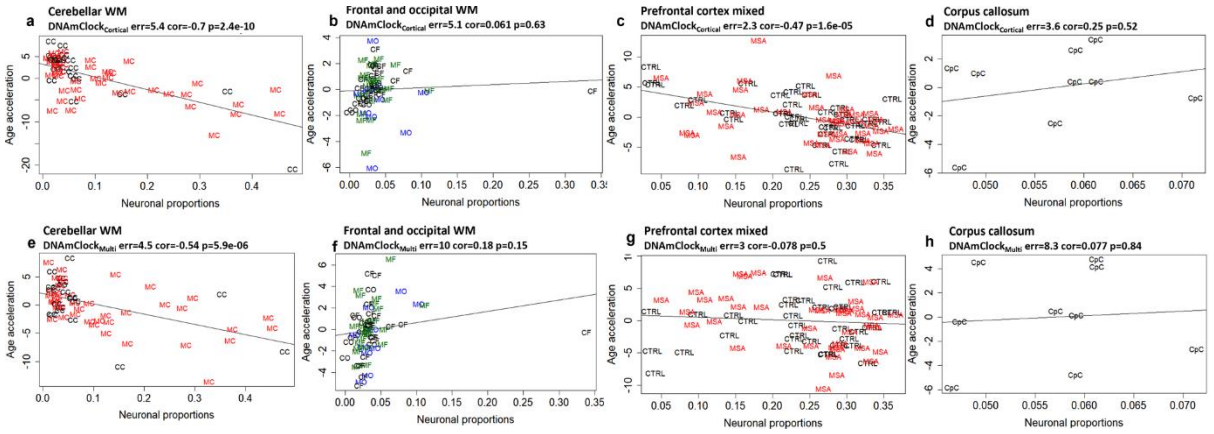

Figure S1. Association between age acceleration and neuronal proportions for DNAmClock$_{Cortical}$ and DNAmClock$_{Multi}$ in the different brain regions. (a-d) Age acceleration residuals (y-axis) versus neuronal proportions (x-axis) for DNAmClock$_{Cortical}$ and **(e-h)** Age acceleration residuals (y-axis) versus neuronal proportions (x-axis) for DNAmClock$_{Multi}$ in the different brain regions. Age acceleration residuals were obtained by regressing DNA methylation age against confounding factors, including chronological age; neuronal proportions were obtained using a DNA methylation-based cell-type deconvolution algorithm. The correlation coefficient and p-values shown were calculated using Pearson correlation. CC – control cerebellum (WM); MC – multiple system atrophy (MSA) cerebellum (WM); CF – control frontal lobe (WM); MF – MSA frontal lobe (WM); CO – control occipital lobe (WM); MO – MSA occipital lobe (WM); PFC – prefrontal cortex (GM+WM), CpC – Corpus callosum; WM – white matter; GM – grey matter.

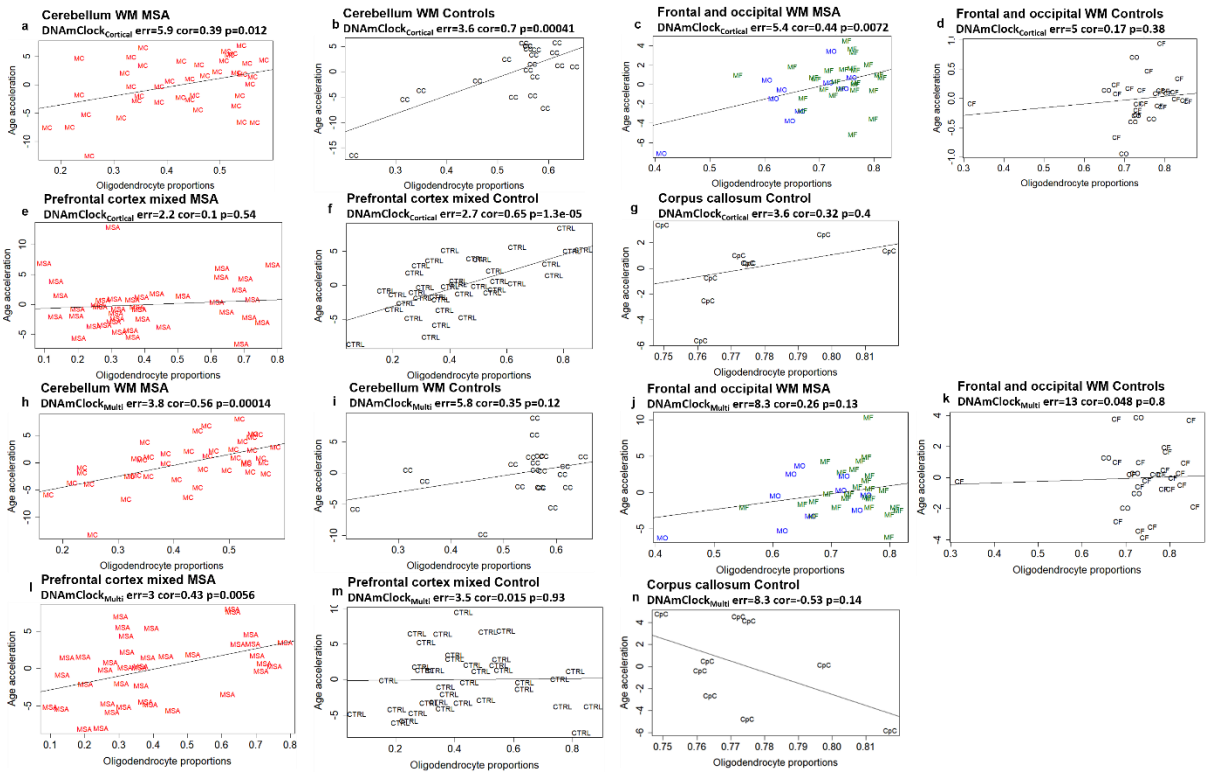

Figure S2. Association between age acceleration and oligodendrocyte proportions for the multiple system atrophy (MSA) cases and control groups for DNAmClock$_{Cortical}$ and DNAmClock$_{Multi}$ in the different brain regions (a-g) Age acceleration residuals (y-axis) versus oligodendrocyte (SOX10+) proportions (x-axis) for the DNAmClock$_{Cortical}$ and **(h-n)** Age acceleration residuals (y-axis) versus oligodendrocyte (SOX10+) proportions (x-axis) for the DNAmClock$_{Multi}$ in the different brain regions. Age acceleration residuals were obtained by regressing DNA methylation age against confounding factors, including chronological age; oligodendrocyte proportions were obtained using a DNA methylation-based cell-type deconvolution algorithm. The correlation coefficient and p-values shown were calculated using Pearson correlation. CC – control cerebellum (WM); MC – MSA cerebellum (WM); CF – control frontal lobe (WM); MF – MSA frontal lobe (WM); CO – control occipital lobe (WM); MO – MSA occipital lobe (WM); PFC - prefrontal cortex (GM+WM); CpC – Corpus callosum; WM – white matter; GM – grey matter.

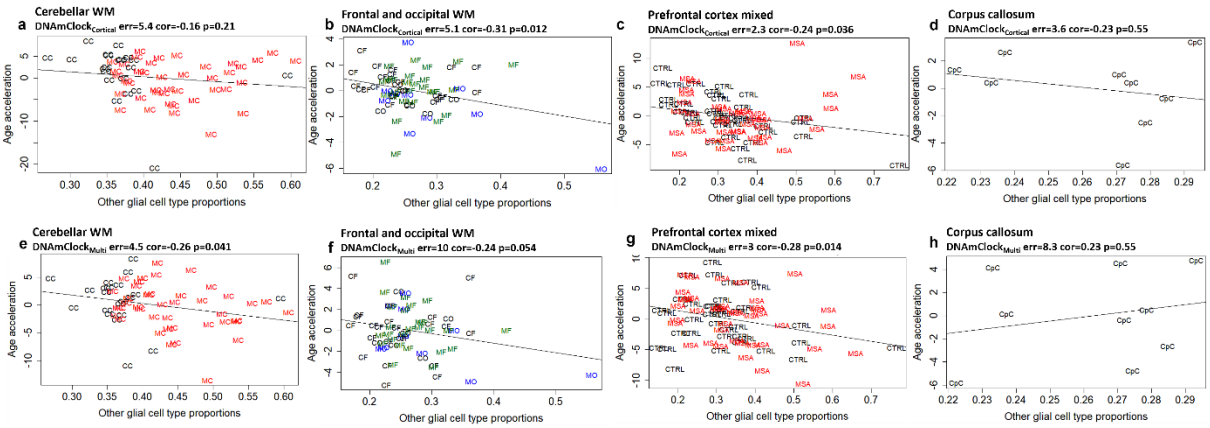

Figure S3. Association between age acceleration and other glial cell type (NeuN-/SOX10-) populations for DNAmClock$_{Cortical}$ and DNAmClock$_{Multi}$ clocks in the different brain regions. (a-d) Age acceleration residuals (y-axis) versus other brain cell type (NeuN-/SOX10-) proportions (x-axis) for the DNAmClock$_{Cortical}$ and **(e-h)** Age acceleration residuals (y-axis) versus other brain cell type (NeuN-/SOX10-) proportions (x-axis) for the DNAmClock$_{Multi}$ in the different brain regions. Age acceleration residuals were obtained by regressing DNA methylation age against confounding factors, including chronological age; NeuN-/SOX10- proportions were obtained using a DNA methylation-based cell-type deconvolution algorithm. The correlation coefficient and p-values shown were calculated using Pearson correlation. CC – control cerebellum (WM); MC – multiple system atrophy (MSA) cerebellum (WM); CF – control frontal lobe (WM); MF – MSA frontal lobe (WM); CO – control occipital lobe (WM); MO – MSA occipital lobe (WM); PFC - prefrontal cortex (GM+WM), CpC – Corpus callosum; WM – white matter; GM – grey matter.

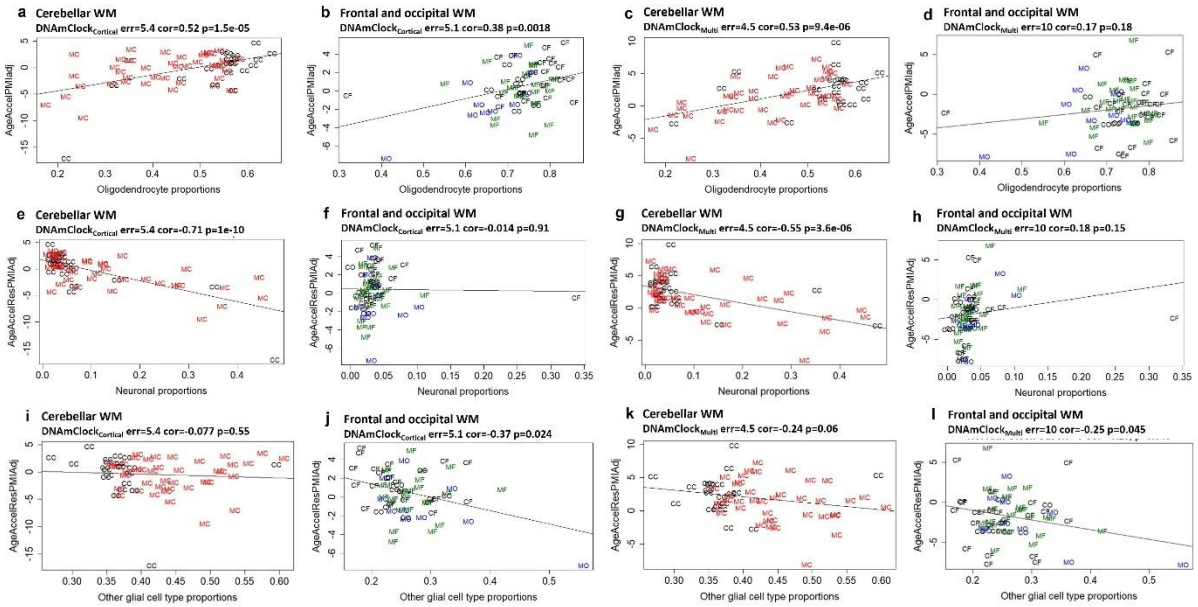

Figure S4. Association between age acceleration and the different cell type proportions for DNAmClock$_{Cortical}$ and DNAmClock$_{Multi}$ in the different brain regions after including post-mortem interval (PMI) as a covariate in cohort 1 regression models. (a-d) Age acceleration residuals (y-axis) versus oligodendrocyte (NeuN-/SOX10+) proportions (x-axis) for both clocks; **(e-h)** age acceleration residuals (y-axis) versus neuronal (NeuN+/SOX10-) proportions (x-axis) for both clocks; **(i-l)** age acceleration residuals (y-axis) versus other glial cell type (NeuN-/SOX10-) proportions (x-axis) for both clocks. Age acceleration residuals were obtained by regressing DNA methylation age against confounding factors, including chronological age; cell proportions were obtained using a DNA methylation-based cell-type deconvolution algorithm. The correlation coefficient and p-values shown were calculated using Pearson correlation. CC – control cerebellum (WM); MC – multiple system atrophy (MSA) cerebellum (WM); CF – control frontal lobe (WM); MF – MSA frontal lobe (WM); CO – control occipital lobe (WM); MO – MSA occipital lobe (WM); WM – white matter.

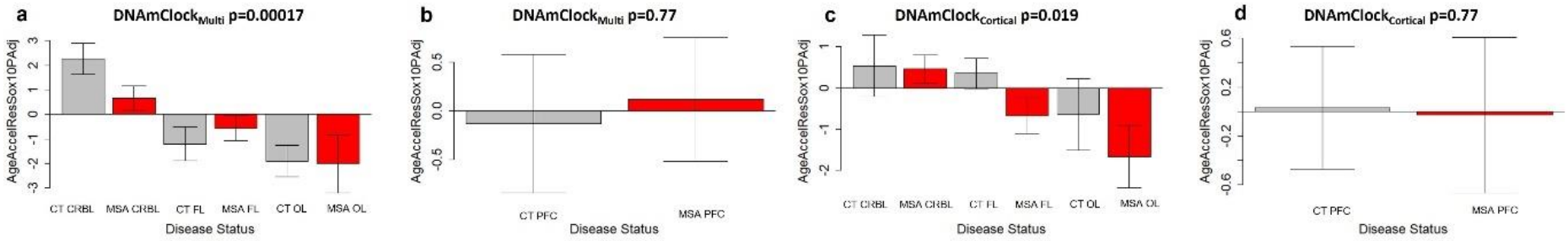

Figure S5. Age acceleration estimation after adjusting for oligodendrocyte proportions for DNAmClock$_{Cortical}$ and DNAmClock$_{Multi}$ in the different brain regions (a-d) Acceleration residual after adjusting for chronological age and oligodendrocyte proportions for the DNAmClock$_{Multi}$ (a - cohort 1; b - cohort 2) and DNAmClock$_{Cortical}$ (c - cohort 1; d - cohort 2) in the different brain regions. The p-values for across group comparisons were calculated using the Kruskal-Wallis test and p-values for pairwise analysis between MSA and controls for each brain region were calculated using the Wilcoxon's test with Benjamini-Hochberg correction for multiple testing. CT CRBL – control cerebellum (WM); MSA CRBL – multiple system atrophy (MSA) cerebellum (WM); CT FL – control frontal lobe (WM); MSA FL – MSA frontal lobe (WM); CT OL – control occipital lobe (WM); MSA OL – MSA occipital lobe (WM); CT PFC – control prefrontal cortex (GM+WM); MSA PFC – MSA prefrontal cortex (GM+WM); WM – white matter; GM – grey matter.

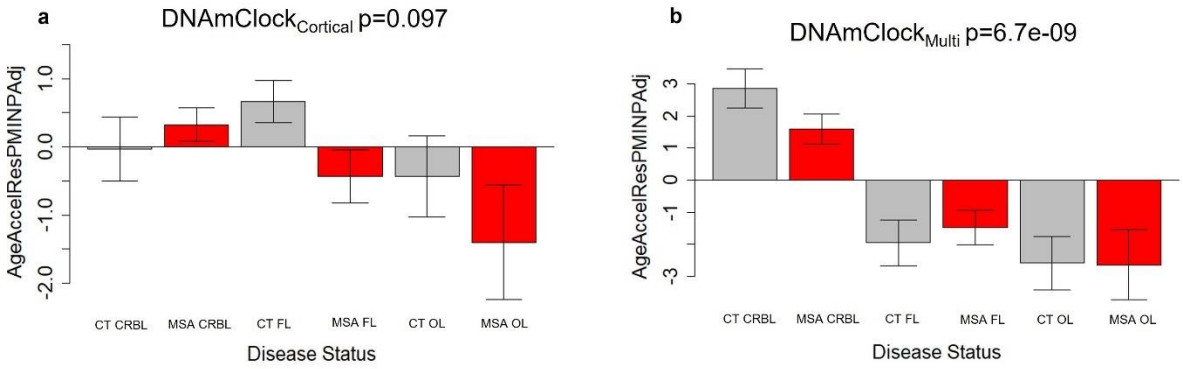

Figure S6. Age acceleration estimation for cohort 1 in the different brain regions for (a) DNAmClock$_{Cortical}$ and (b) DNAmClock$_{Multi}$ after adjusting for post-mortem interval (PMI) (Chronological age, neuronal proportions, replicate individuals and PMI were included in the regression model). CT CRBL - control cerebellum (WM); MSA CRBL – multiple system atrophy (MSA) cerebellum (WM); CT FL – control frontal lobe (WM); MSA FL – MSA frontal lobe (WM); CT OL – control occipital lobe (WM); MSA OL – MSA occipital lobe (WM); CT PFC – control prefrontal cortex (GM+WM); MSA PFC – MSA prefrontal cortex (GM+WM); WM – white matter; GM – grey matter. The p-values for across group comparisons were calculated using the Kruskal-Wallis test and p-values for pairwise analysis between MSA and controls for each brain region were calculated using the Wilcoxon's test with Benjamini-Hochberg correction for multiple testing.

Table S1. Cohort demographics.

Tissue/Region	Controls					MSA				
	No. of samples			Mean Chronological age ±SD		No. of samples			Mean Chronological age ±SD	
Cohort 1	**N**	**Females (%)**	**Males (%)**	**Females**	**Males**	**N**	**Females (%)**	**Males (%)**	**Females**	**Males**
Cerebellum (WM)	21	47.6	52.4	81.1±9	79.55±9	41	48.8	51.2	64.2±7	64.48±8
Frontal lobe (WM)	23	47.8	52.2	76±10	76.58±7	26	50	50	65.85±4	67.08±8
Occipital lobe (WM)	6	33.3	66.7	95±5	83.75±8	10	50	50	64.6±3	63.4±8
Cohort 2										
Prefrontal cortex (GM+WM)	37	48.6	51.4	72.78±9	73.16±11	40	56.1	41.5	64.91±5	67.47±6
Cohort 3										
Corpus callosum	9	22.2	77.8	58±9	61.43±13	-	-	-	-	-
Cohort 4										
Sox10+ nuclei	15	53.3	46.7	77.88±12	81.86±8	-	-	-	-	-

MSA - multiple system atrophy; WM - white matter; GM+WM - Mix of grey and white matter; SD - Standard deviation

Table S2. Difference in median absolute deviation between controls and MSA for $DNAmClock_{Multi}$ and $DNAmClock_{Cortical}$.

Region	$DNAmClock_{Cortical}$			$DNAmClock_{Multi}$		
	CTRL	**MSA**	**p value (two.sided)**	**CTRL**	**MSA**	**p value (two.sided)**
Cohort 1						
Cerebellum (WM)	3.6	5.9	0.158	5.8	3.8	0.014
Frontal lobe (WM)	5.1	5.4	0.912	12	8.3	0.016
Occipital lobe (WM)	2.6	5.3	0.626	16	8.7	0.008
Cohort 2						
Prefrontal cortex (GM+WM)	2.7	3.5	0.614	2.2	3	0.795
Total			**0.222**			**0.011**

CTRL - control; MSA - multiple system atrophy; WM - white matter; GM+WM - Mix of grey and white matter; the p-values were calculated using two-sided Wilcoxon rank sum test with continuity correction.

Table S3. Mean chronological and DNAm ages for $DNAmClock_{Multi}$ and $DNAmClock_{Cortical}$ for all brain regions of control and MSA samples.

Tissue/Region	N		Mean chronological age		Mean DNAm age			
					$DNAmClock_{Cortical}$		$DNAmClock_{Multi}$	
	CTRL	MSA	CTRL	MSA	CTRL	MSA	CTRL	MSA
Cohort 1								
Cerebellum (WM)	21	41	80.28	64.34	81.1	68.9	72.53	60.26
Frontal lobe (WM)	23	26	76.3	66.46	80.3	71.8	64.06	58.3
Occipital lobe (WM)	6	10	87.5	64	84.2	66.98	69.8	54.9
Cohort 2								
Prefrontal cortex (GM+WM)	37	41	72.97	66	73.26	66.66	71.27	66.41
Cohort 3								
Corpus callosum	9		60.66		57.03		53.17	
Cohort 4								
SOX10+ nuclei	15		79.73		90.11		53.74	

CTRL - control; MSA - multiple system atrophy; WM - white matter; GM+WM - Mix of grey and white matter