Supplementary Material

Title: Altered dynamics of glymphatic flow in a mature-onset Tet-off APP mouse model of amyloidosis

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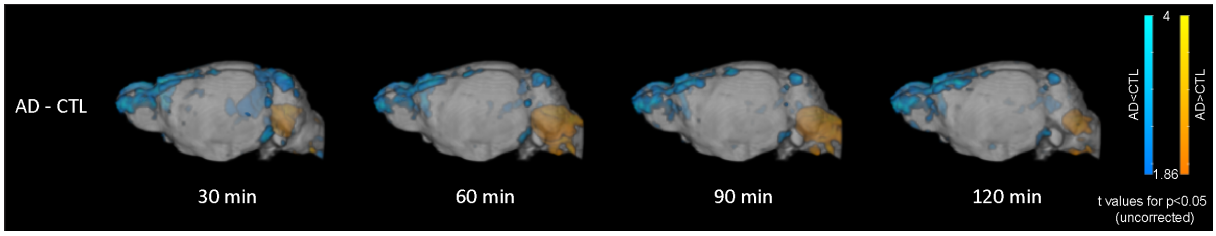
Voxel-based analysis reflects differences in the distribution of Gd-DOTA across groups.

To assess the differences in the spatial distribution of the Gd-DOTA between the CTL and AD groups, voxel-based analysis (VBA) was performed at four time-points after injection (30, 60, 90, 120 min). To this end, a two-sample *t*-test was performed on a voxel-by-voxel basis comparing the percent signal change from baseline across the CTL and AD groups. No statistical significance was observed after multiple comparison correction, but Additional Figure 1 presents uncorrected statistics ($p<0.05$, uncorrected) that indicate a general agreement with our ROI-based and cluster-based analyses. Specifically, percent signal change was higher in the AD group in areas nearby the injection cite (pons, medulla; orange/yellow) as also observed in other analyses. In addition, VBA revealed signal decreases in AD relative to CTL in caudal areas close to the superior sagittal and transverse sinuses (blue) but note that signal intensity in those areas was low and within the variability observed in the non-injected groups and thus it is difficult to make robust conclusions.



Additional Figure 1. Voxel-based analysis (VBA). *The signal intensity of CTL and AD mice were compared at four time-points after injection (30, 60, 90, 120 min). The AD group demonstrated stronger signal intensity in regions adjacent to the infusion spot (orange/yellow) in all time points and decreased signal intensity in areas proximal to the superior sagittal and transverse sinuses (blue). The colour scales indicate T-statistic values, with orange/yellow representing the voxels significantly higher in the AD group and blue/cyan representing the voxels significantly higher in the CTL group (p<0.05, uncorrected).*

Supplementary Statistics

Supplementary Table 1: Linear Mixed-Effects model analysis for total GFAP signal

Linear mixed-effects model fit by ML

Model information:

Number of observations	72
Fixed effects coefficients	4
Random effects coefficients	6
Covariance parameters	2

Formula:

Mean ~ 1 + Brain_area + mouse_type + (1 | mouse_id)

Model fit statistics:

AIC	BIC	LogLikelihood	Deviance
603.48	617.14	−295.74	591.48

Fixed effects coefficients (95% CIs):

Name	Estimate	SE	tStat	DF	pValue	Lower	Upper
{'(Intercept)' }	62.176	3.0028	20.706	68	4.486e−31	56.184	68.168
{'Brain_area_CTX' }	0.44392	4.2466	0.10453	68	0.91705	−8.03	8.9179
{'Brain_area_Amygdala'}	−18.874	4.2466	−4.4444	68	3.3347e−05	−27.348	−10.4
{'mouse_type_WT' }	−18.005	3.4673	−5.1926	68	2.0482e−06	−24.924	−11.086

Random effects covariance parameters (95% CIs):

Group: mouse_id (6 Levels)

Name1	Name2	Type	Estimate	Lower	Upper
{'(Intercept)'}	{'(Intercept)'}	{'std'}	1.6332e−15	NaN	NaN

Group: Error

Name	Estimate	Lower	Upper
{'Res Std'}	14.711	12.494	17.321

Supplementary Table 2: Linear Mixed-Effects model analysis for vessel coverage

Linear mixed-effects model fit by ML

Model information:

Number of observations	73
Fixed effects coefficients	4
Random effects coefficients	6
Covariance parameters	2

Formula:

coverage ~ 1 + group + area + (1 | id)

Model fit statistics:

AIC	BIC	LogLikelihood	Deviance
−94.209	−80.467	53.105	−106.21

Fixed effects coefficients (95% CIs):

Name	Estimate	SE	tStat	DF	pValue	Lower	Upper
{'(Intercept)' }	0.51311	0.023811	21.549	69	2.3339e−32	0.46561	0.56061
{'group_WT' }	0.071953	0.027375	2.6284	69	0.010565	0.017341	0.12656
{'area_CTX' }	−0.059394	0.033158	−1.7913	69	0.077636	−0.12554	0.0067536
{'area_Amygdala'}	−0.056074	0.033747	−1.6616	69	0.10113	−0.1234	0.01125

Random effects covariance parameters (95% CIs):

Group: id (6 Levels)

Name1	Name2	Type	Estimate	Lower	Upper
{'(Intercept)'}	{'(Intercept)'}	{'std'}	2.5958e−17	NaN	NaN

Group: Error

Name	Estimate	Lower	Upper
{'Res Std'}	0.1169	0.099399	0.13749

Supplementary Table 3: Linear Mixed-Effects model analysis for vascular density

Linear mixed-effects model fit by ML

Model information:

Number of observations	18
Fixed effects coefficients	2
Random effects coefficients	6
Covariance parameters	2

Formula:

x_Area ~ 1 + mouse_type + (1 | mouse_id)

Model fit statistics:

AIC	BIC	LogLikelihood	Deviance
60.651	64.212	−26.325	52.651

Fixed effects coefficients (95% CIs):

Name	Estimate	SE	tStat	DF	pValue	Lower	Upper
{'(Intercept)' }	4.7046	0.50422	9.3303	16	7.1419e−08	3.6357	5.7735
{'mouse_type_WT'}	0.69578	0.71308	0.97574	16	0.34372	−0.81588	2.2074

Random effects covariance parameters (95% CIs):

Group: mouse_id (6 Levels)

Name1	Name2	Type	Estimate	Lower	Upper
{'(Intercept)'}	{'(Intercept)'}	{'std'}	0.71525	0.30083	1.7006

Group: Error

Name	Estimate	Lower	Upper
{'Res Std'}	0.86799	0.58179	1.295