

Microglia modulate A β -dependent astrocyte reactivity in Alzheimer's disease

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Supplementary Information

Article: “Microglia modulate A β -dependent astrocyte reactivity in Alzheimer’s disease”.

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Abbreviations: AD = Alzheimer’s disease; AHBA = Allen Human Brain Atlas; *APOE* ϵ 4 = Apolipoprotein E ϵ 4; A β = amyloid- β ; CFI = comparative fit index; CI = confidence interval; CSF = cerebrospinal fluid; CU = cognitively unimpaired; FDR = false discovery rate; GFAP = glial fibrillary acidic protein; IQR = interquartile range; MA = microglial activation; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; PET = positron emission tomography; p-tau₂₁₇ = phosphorylated tau at threonine 217; RMSEA = root mean squared error of approximation; ROI = region of interest; SD = standard deviation; SRMR = standardized root mean square residual; sTREM2 = soluble triggering receptor expressed on myeloid cells 2; SUVR = standardized uptake value ratio; TRIAD = Translational Biomarkers in Aging and Dementia; TSPO = translocator protein; WRAP = Wisconsin Registry for Alzheimer’s Prevention.

Supplementary Table 1 | Demographic characteristics of the primary sample according to MA_{TSPO} status.

	Overall	MA-TSPO	MA+TSPO
No.	101	50	51
Age, years	71.9 (6.5)	71.4 (5.7)	72.4 (7.2)
Male, No. (%)	33 (32.7)	11 (22.0)	22 (43.1)
Education, years	15.3 (3.6)	15.3 (3.8)	15.3 (3.5)
<i>APOE</i> ε4 carrier, No. (%)	35 (34.7)	16 (32.0)	19 (37.3)
Plasma GFAP, pg/mL	242.3 (119.8)	224.9 (110.0)	259.4 (127.4)
Plasma p-tau217, pg/mL ^a	0.09 (0.08)	0.07 (0.06)	0.14 (0.15)
Aβ PET SUVR	1.74 (0.60)	1.61 (0.56)	1.87 (0.62)
Tau PET SUVR	1.13 (0.71)	0.99 (0.41)	1.27 (0.89)
TSPO PET SUVR	1.31 (0.14)	1.20 (0.08)	1.41 (0.10)
Cognitively impaired, No. (%) ^b	39 (38.6)	17 (34.0)	22 (43.1)
MMSE score	28.2 (2.8)	28.7 (1.9)	27.7 (3.4)

Continuous variables are presented as mean (SD). Aβ PET was measured with global [¹⁸F]AZD4694 SUVR, TSPO PET with posterior cingulate [¹¹C]PBR28 SUVR, and tau PET with temporal meta-ROI [¹⁸F]MK6240 SUVR. ^aAssessed in a subset of 93 individuals. ^bComposed of 26 individuals with MCI and 13 individuals with AD dementia.

Supplementary Table 2 | Demographic characteristics of the secondary sample.

	Overall	MA ⁻ sTREM2	MA ⁺ sTREM2
No.	251	116	135
Age, years	67.2 (7.3)	65.9 (7.3)	68.3 (7.2)
Male, No. (%)	95 (37.8)	42 (36.2)	53 (39.3)
Education, years	15.6 (2.9)	15.9 (2.6)	15.3 (3.01)
<i>APOE</i> ε4 carrier, No. (%) ^a	95 (37.8)	49 (42.2)	46 (34.1)
Plasma GFAP (z-score)	0.36 (1.24)	0.39 (1.24)	0.33 (1.25)
Aβ PET centiloid	20.9 (32.8)	24.4 (36.2)	17.8 (29.4)
CSF sTREM2 (z-score)	-0.08 (1.03)	-0.93 (0.52)	0.65 (0.77)
Cognitively impaired, No. (%) ^b	37 (14.7)	13 (11.2)	24 (17.8)
MMSE score	29.1 (1.2)	29.2 (1.0)	28.9 (1.3)

Continuous variables are presented as mean (SD). Global Aβ PET burden was assessed with [¹¹C]PiB (WRAP) and [¹⁸F]AZD4694 (TRIAD) in the centiloid scale. Fluid biomarker levels were standardized using the mean and SD of each cohort's CU Aβ- subset. Of the 251 individuals in the secondary cohort, 150 (59.8%) were WRAP participants and 101 (40.2%) were TRIAD participants. ^aInformation unavailable for 3 MA⁻sTREM2 individuals from the WRAP cohort.

^bComposed of MCI participants.

Supplementary Table 3 | Time differences between imaging, fluid, and cognitive data in the primary sample.

	Median (IQR) time difference, years
A β PET and TSPO PET	0.21 (0.33)
A β PET and plasma GFAP	0.37 (0.36)
A β PET and plasma p-tau217	0.40 (0.52)
A β PET and tau PET	0.12 (0.23)
A β PET and MMSE	0.14 (0.37)
TSPO PET and plasma GFAP	0.44 (0.21)
TSPO PET and plasma p-tau217	0.43 (0.23)
TSPO PET and tau PET	0.19 (0.27)
TSPO PET and MMSE	0.24 (0.33)
Plasma GFAP and plasma p-tau217	0 (0)
Plasma GFAP and tau PET	0.43 (0.44)
Plasma GFAP and MMSE	0.25 (0.42)
Plasma p-tau217 and tau PET	0.40 (0.43)
Plasma p-tau217 and MMSE	0.33 (0.50)
Tau PET and MMSE	0.19 (0.35)

Supplementary Table 4 | Time differences between imaging and fluid data in the secondary sample.

	Median (IQR) time difference, years	
	WRAP	TRIAD
A β PET and CSF sTREM2	0.02 (0.39)	0.17 (0.28)
A β PET and plasma GFAP	0.27 (0.65)	0.32 (0.34)
CSF sTREM2 and plasma GFAP	0.12 (0.48)	0.21 (0.26)

Supplementary Table 5 | FDR-adjusted *P*-values and associated statistics for Fig. 1a,b.

	β (95% CI)	<i>T</i> -value	FDR-adjusted <i>P</i> -value
MA⁻TSPO: Plasma GFAP ~ Aβ PET + age + sex + cognitive status			
A β PET	0.185 (-0.133 to 0.502)	1.173	0.247
MA⁺TSPO: Plasma GFAP ~ Aβ PET + age + sex + cognitive status			
A β PET	0.618 (0.310 to 0.926)	4.040	< 0.001
All sample: Plasma GFAP ~ Aβ PET + MA_{TSPO} status + age + sex + cognitive status + Aβ PET x MA_{TSPO} status			
A β PET x MA ⁺ TSPO	0.528 (0.172 to 0.883)	2.945	0.005
All sample: Plasma GFAP ~ Aβ PET + TSPO PET + age + sex + cognitive status + Aβ PET x TSPO PET			
A β PET x TSPO PET	0.302 (0.116 to 0.489)	3.217	0.004

β -estimates and corresponding statistics were computed from linear regression models. Continuous biomarker levels were standardized prior to model entry. A β PET was measured with global [¹⁸F]AZD4694 SUVR, and TSPO PET with posterior cingulate [¹¹C]PBR28 SUVR.

Supplementary Table 6 | Sensitivity analyses testing the association of TSPO PET and A β PET with plasma GFAP including outliers.

	β (95% CI)	<i>T</i>-value	<i>P</i>-value
MA_{-TSPO}: Plasma GFAP ~ Aβ PET + age + sex + cognitive status			
A β PET	0.258 (-0.070 to 0.586)	1.585	0.120
MA_{+TSPO}: Plasma GFAP ~ Aβ PET + age + sex + cognitive status			
A β PET	0.560 (0.281 to 0.839)	4.040	< 0.001
All sample: Plasma GFAP ~ Aβ PET + MA_{TSPO} status + age + sex + cognitive status + Aβ PET x MA_{TSPO} status			
A β PET x MA _{+TSPO}	0.376 (0.030 to 0.722)	2.157	0.034
All sample: Plasma GFAP ~ Aβ PET + TSPO PET + age + sex + cognitive status + Aβ PET x TSPO PET			
A β PET x TSPO PET	0.244 (0.062 to 0.426)	2.663	0.009

β -estimates and corresponding statistics were computed from linear regression models. Continuous biomarker levels were standardized prior to model entry. A β PET was measured with global [^{18}F]AZD4694 SUVR, and TSPO PET with posterior cingulate [^{11}C]PBR28 SUVR. This analysis was conducted including the two outliers (plasma GFAP levels three SD above the mean of the population) excluded from the main analyses.

Supplementary Table 7 | FDR-adjusted *P*-values and associated statistics for Fig. 2a,b.

	β (95% CI)	<i>T</i> -value	FDR-adjusted <i>P</i> -value
MA⁻TSPO: Plasma p-tau217 ~ plasma GFAP + age + sex + cognitive status			
Plasma GFAP	0.110 (-0.091 to 0.312)	1.102	0.276
MA⁺TSPO: Plasma p-tau217 ~ plasma GFAP + age + sex + cognitive status			
Plasma GFAP	0.658 (0.323 to 0.993)	3.973	0.001
All sample: Plasma p-tau217 ~ plasma GFAP + MA_{TSPO} status + age + sex + cognitive status + plasma GFAP x MA_{TSPO} status			
Plasma GFAP x MA ⁺ TSPO	0.471 (0.125 to 0.816)	2.709	0.016
All sample: Plasma p-tau217 ~ plasma GFAP + TSPO PET + age + sex + cognitive status + plasma GFAP x TSPO PET			
Plasma GFAP x TSPO PET	0.201 (0.024 to 0.377)	2.264	0.035

β -estimates and corresponding statistics were computed from linear regression models. Continuous biomarker levels were standardized prior to model entry. A β PET was measured with global [¹⁸F]AZD4694 SUVR, and TSPO PET with posterior cingulate [¹¹C]PBR28 SUVR.

Supplementary Table 8 | Sensitivity analyses testing the association of TSPO PET and plasma GFAP with plasma p-tau217 including outliers.

	β (95% CI)	<i>T</i>-value	<i>P</i>-value
MA-TSPO: Plasma p-tau217 ~ plasma GFAP + age + sex + cognitive status			
Plasma GFAP	0.063 (-0.065 to 0.191)	0.996	0.325
MA+TSPO: Plasma p-tau217 ~ plasma GFAP + age + sex + cognitive status			
Plasma GFAP	0.791 (0.484 to 1.098)	5.199	< 0.001
All sample: Plasma p-tau217 ~ plasma GFAP + MA_{TSPO} status + age + sex + cognitive status + plasma GFAP x MA_{TSPO} status			
Plasma GFAP x MA _{TSPO}	0.652 (0.348 to 0.957)	4.254	< 0.001
All sample: Plasma p-tau217 ~ plasma GFAP + TSPO PET + age + sex + cognitive status + plasma GFAP x TSPO PET			
Plasma GFAP x TSPO PET	0.299 (0.132 to 0.465)	3.567	< 0.001

β -estimates and corresponding statistics were computed from linear regression models. Continuous biomarker levels were standardized prior to model entry. A β PET was measured with global [¹⁸F]AZD4694 SUVR, and TSPO PET with posterior cingulate [¹¹C]PBR28 SUVR. This analysis was conducted including the five outliers (plasma GFAP or p-tau217 levels three SD above the mean of the population) excluded from the main analyses.

Supplementary Table 9 | Structural equation model coefficients and associated statistics for Fig. 2d.

	Std. β (95% CI)	Z-value	P-value
MMSE^a			
Temporal meta-ROI tau PET	-0.154 (-0.455 to 0.147)	-1.005	0.315
Plasma p-tau217	-0.025 (-0.388 to 0.337)	-0.137	0.891
Plasma GFAP	-0.179 (-0.426 to 0.068)	-1.420	0.156
A β PET	-0.315 (-0.715 to 0.084)	-1.549	0.121
Temporal meta-ROI tau PET^b			
Plasma p-tau217	-0.004 (-0.340 to 0.331)	-0.024	0.981
Plasma GFAP	-0.034 (-0.265 to 0.196)	-0.291	0.771
A β PET	0.635 (0.347 to 0.924)	4.311	< 0.001
Plasma p-tau217^c			
Plasma GFAP	0.033 (-0.159 to 0.226)	0.340	0.734
A β PET	0.722 (0.604 to 0.840)	11.977	< 0.001
Plasma GFAP^d			
A β PET	0.127 (-0.136 to 0.391)	0.947	0.343

Structural equation modeling testing the associations between A β (global [^{18}F]AZD4694 A β PET), reactive astrogliosis (plasma GFAP), tau phosphorylation (plasma p-tau217), tau tangles (temporal meta-ROI [^{18}F]MK6240 tau PET), and cognition (MMSE) in MA-TSPO individuals. Associations were adjusted for age, sex, and (if involving cognition) years of education. The model fit the data poorly ($n = 49$, $X^2 = 12.672$, degrees of freedom = 3, $P = 0.005$, RMSEA = 0.257, SRMR = 0.069, CFI = 0.888). ^aR²: 0.32; ^bR²: 0.41; ^cR²: 0.59; ^dR²: 0.13.

Supplementary Table 10 | Structural equation model coefficients and associated statistics for Fig. 2e.

	Std. β (95% CI)	Z-value	P-value
MMSE^a			
Temporal meta-ROI tau PET	-0.813 (-1.017 to -0.610)	-7.845	< 0.001
Plasma p-tau217	-0.039 (-0.306 to 0.229)	-0.283	0.777
Plasma GFAP	0.161 (-0.058 to 0.380)	1.441	0.150
A β PET	-0.142 (-0.389 to 0.106)	-1.123	0.262
Temporal meta-ROI tau PET^b			
Plasma p-tau217	0.519 (0.228 to 0.810)	3.494	< 0.001
Plasma GFAP	-0.141 (-0.414 to 0.132)	-1.014	0.310
A β PET	0.321 (0.028 to 0.613)	2.146	0.032
Plasma p-tau217^c			
Plasma GFAP	0.297 (0.049 to 0.545)	2.349	0.019
A β PET	0.576 (0.365 to 0.786)	5.369	< 0.001
Plasma GFAP^d			
A β PET	0.615 (0.462 to 0.768)	7.871	< 0.001

Structural equation modeling testing the associations between A β (global [¹⁸F]AZD4694 A β PET), reactive astrogliosis (plasma GFAP), tau phosphorylation (plasma p-tau217), tau tangles (temporal meta-ROI [¹⁸F]MK6240 tau PET), and cognition (MMSE) in MA⁺_{TSP0} individuals. Associations were adjusted for age, sex, and (if involving cognition) years of education. The model fit the data well ($n = 44$, $X^2 = 0.856$, degrees of freedom = 3, $P = 0.836$, RMSEA = 0.000, SRMR = 0.018, CFI = 1.000). ^aR²: 0.76; ^bR²: 0.61; ^cR²: 0.63; ^dR²: 0.49.

Supplementary Table 11 | Demographics of the CU young population.

	Sample used to calculate the threshold for TSPO PET	Sample used to calculate the threshold for CSF sTREM2
No.	17	24
Age, years	22.4 (1.3)	22.5 (1.1)
Male, No. (%)	8 (47.1)	10 (41.7)
Education, years	16.4 (1.5)	16.5 (1.4)
<i>APOE</i> ε4 carrier, No. (%)	2 (11.8)	6 (25.0)
Plasma GFAP, pg/mL	77.9 (35.9)	84.9 (69.2)
Aβ PET SUVR	1.20 (0.06)	1.21 (0.06)
TSPO PET SUVR	1.14 (0.08)	1.13 (0.08) ^a
CSF sTREM2, pg/mL	1388.7 (615.8) ^a	1379.8 (572.1)
MMSE score	29.8 (0.5)	29.8 (0.5)

Continuous variables are presented as mean (SD). Aβ PET was measured with global [¹⁸F]AZD4694 SUVR, and TSPO PET with posterior cingulate [¹¹C]PBR28 SUVR. All CU young individuals are from the TRIAD cohort. ^aAssessed in a subset of 15 individuals overlapping between the CU young samples used to determine the TSPO PET and CSF sTREM2 thresholds.

Supplementary Table 12 | Demographics for AHBA donors.

	Donors^a
No.	6
Age, years	42.5 (13.4)
Male, No. (%)	5 (83.3)
Postmortem Interval, hours ^b	20.7 (7.0)

Continuous variables are presented as mean (SD). ^aDonors included in the Microarray Survey study of the AHBA had no known history of neuropsychiatric or neurological conditions. ^bEstimated time from death to the time that tissue was frozen.