

## Brain-age prediction and its associations with glial and synaptic CSF markers

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ALFA study

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**Background:** MRI-derived brain-age prediction is a promising biomarker of biological brain aging. Accelerated brain aging has been found in Alzheimer's disease (AD) and other neurodegenerative diseases. However, no previous studies have investigated the relationship between specific pathophysiological pathways in

AD and biological brain aging. Here, we studied whether glial activation and synaptic dysfunction are associated with biological brain aging in the earliest stages of the Alzheimer's continuum.

**Methods:** We included 418 cognitively unimpaired individuals (CU) from the ALFA+ study with available structural MRI, and CSF biomarkers of amyloid- $\beta$  ( $A\beta_{42/40}$ ) and tau pathology (p-tau181), synaptic dysfunction (neurogranin, GAP43, SYT1, SNAP25), glial activation (sTREM2, YKL40, GFAP, interleukin-6 and S100b) and  $\alpha$ -synuclein (Table 1).  $A\beta_{42/40}$ , neurogranin and the glial activation biomarkers were measured using the Roche NeuroToolKit. We computed brain-age delta as the difference between chronological and predicted brain-age. The latter was estimated using a previously pretrained machine learning algorithm on cerebral morphological measurements on individuals from the UKBioBank cohort (N=22.000). General linear modeling was used to test the associations between CSF biomarkers and brain-age delta, adjusting by p-tau, age, *APOE* status and sex. For the biomarkers whose associations were significant, we evaluated the interaction term "biomarker"  $\times$  AT status while adjusting by age, *APOE* status and sex. AT staging was performed using pre-established cut-off values. We then used hippocampal volume as a marker of AD-related neurodegeneration and repeated the same association studies with CSF biomarkers, adjusting by p-tau, age, *APOE* status, sex and TIV.

**Result:** Brain-age delta was negatively associated with CSF sTREM2 ( $P_{\text{adjusted}} < 0.001$ ), meaning that younger-appearing brains showed higher levels of this biomarker (Table 1). None of the other biomarkers survived multiple

comparisons. Hippocampal volume was not significantly associated with any of the CSF biomarkers (Table 2). There was no significant interaction between AT status and CSF sTREM2 for brain-age delta, nor for hippocampal volume.

**Conclusion:** These results showed that higher levels of CSF sTREM2 were associated with younger-appearing brains in CU individuals independently of AT status, which might indicate a protective effect of this microglial phenotype in brain aging in brain aging. This effect might not be AD-related.

**Table 1.** Participants descriptive and characteristics.

	Whole sample (N=380)	A-T- (N=236)	A+T- (N=100)	A+T+ (N=31)	A-T+ (N=13)
Age [years]	61.038 (4.669)	60.428 (4.425)	61.140 (4.515)	63.809 (4.433)	61.140 (4.516)
Females, n (%)	254 (60.765)	145 (61.441)	59 (58.416)	21 (70.00)	9 (69.230)
<i>APOE-e4</i> carr (%)	210 (54.545)	117 (52.94)	49 (54.444)	15 (53.571)	8 (61.538)
Brain-age delta	0.000 (3.671)	0.093 (3.834)	3.110 (4.135)	-4.065 (4.512)	0.311 (4.135)
Amyloid- $\beta$	0.0749 (0.020)	0.0867 (0.009)	0.053 (0.011)	0.043 (0.011)	0.0970 (0.018)
p-tau	16.336 (7.625)	13.873 (4.192)	15.565 (4.067)	33.438 (11.872)	27.589 (4.577)
GAP43	2851.340 (1205.667)	2538.232 (918.159)	2621.347 (685.187)	5028.044 (1360.809)	5065.030 (985.652)
GFAP	7.694 (2.651)	7.365 (2.523)	7.471 (2.179)	10.411 (2.151)	9.149 (4.808)
IL6	4.007 (1.890)	3.942 (1.825)	4.157 (2.132)	4.195 (1.781)	3.623 (1.307)
Neurogranin	803.779 (332.656)	718.246 (250.131)	746.714 (217.742)	1400.433 (346.837)	1422.976 (327.620)
S100b	1.026 (0.236)	0.993 (0.200)	1.053 (0.273)	1.152 (0.268)	1.134 (0.331)
YKL40	147.091 (52.763)	138.562 (43.871)	139.992 (46.342)	212.550 (65.898)	206.033 (74.256)
a-Synuclein	208.383 (22.409)	202.158 (121.514)	180.621 (52.821)	311.411 (93.519)	322.146 (57.924)
SNAP25	21.671 (3.159)	20.925 (2.628)	21.525 (2.616)	26.508 (3.676)	24.920 82.703)
SYT1	52.387 (0.699)	48.934 (10.473)	49.837 (8.723)	75.126 (18.185)	80.579 (11.888)
sTREM2	7.969 (2.267)	7.746 (2.006)	7.502 (2.021)	9.882 (2.727)	11.236 (2.837)

Abbreviations: IL6 = cytokine interleukin-6; GFAP = glial fibrillary acidic protein; GAP-43 = growth-associated protein-43; SNAP25 = synaptosomal-associated protein-25; sTREM2=soluble triggering receptor on myeloid cells 2; S100b = S100 calcium-binding protein b.

**Table 2.** Effect of CSF Synaptic and Glial Biomarkers on brain-age delta

	$\beta$ (SE)	SE	P	[0.025	0.075]	$P_{adjusted}$
GAP43	-0.214	0.099	0.032	-0.409	-0.018	0.107
GFAP	-0.023	0.065	0.719	-0.15	0.104	0.800
IL6	-0.003	0.054	0.952	-0.11	0.104	0.952
Neurogranin	0.049	0.099	0.623	-0.145	0.242	0.778
S100b	-0.123	0.055	0.027	-0.231	-0.014	0.106
YKL40	-0.056	0.074	0.451	-0.202	0.09	0.644
a-Synuclein	-0.020	0.071	0.776	-0.16	0.119	0.816
SNAP25	-0.104	0.082	0.205	-0.264	0.057	0.389
SYT1	-0.256	0.098	0.009	-0.449	-0.063	0.090
sTREM2	-0.227	0.063	<0.001	-0.351	-0.104	<0.001*

Abbreviations: Adj = false discovery rate adjusted; IL6 = cytokine interleukin-6; GFAP = glial fibrillary acidic protein; GAP-43 = growth-associated protein-43; SE = standard error; SNAP25 = synaptosomal-associated protein-25; sTREM2=soluble triggering receptor on myeloid cells 2; S100b = S100 calcium-binding protein b. The associations between each CSF synaptic biomarker and brain-age delta were tested in a linear model in the whole sample. All analyses were adjusted for p-tau, age, sex and *APOE* status. The regression coefficients ( $\beta$ ) and SEs are depicted. All p values are corrected for multiple comparisons using the false discovery rate approach.

**Table 3.** Effect of CSF Synaptic and Glial Biomarkers on hippocampal volume

	$\beta$ (SE)	SE	<i>P</i>	[0.025	0.075]	<i>P</i> <sub>adjusted</sub>
<b>GAP43</b>	0.059	0.077	0.446	-0.093	0.211	0.644
<b>GFAP</b>	0.062	0.050	0.214	-0.036	0.16	0.389
<b>IL6</b>	-0.073	0.042	0.083	-0.156	0.01	0.225
<b>Neurogranin</b>	-0.058	0.076	0.446	-0.208	0.092	0.644
<b>S100b</b>	0.104	0.043	0.016	0.02	0.188	0.107
<b>YKL40</b>	-0.075	0.057	0.193	-0.187	0.038	0.389
<b>a-Synuclein</b>	0.032	0.055	0.558	-0.076	0.141	0.744
<b>SNAP25</b>	0.023	0.063	0.712	-0.1	0.146	0.800
<b>SYT1</b>	0.129	0.076	0.090	-0.02	0.277	0.225
<b>sTREM2</b>	0.111	0.049	0.024	0.014	0.207	0.107

**Abbreviations:** Adj = false discovery rate adjusted; IL6 = cytokine interleukin-6; GFAP = glial fibrillary acidic protein; GAP-43 = growth-associated protein-43; SE = standard error; SNAP25 = synaptosomal-associated protein-25; sTREM2=soluble triggering receptor on myeloid cells 2; S100b = S100 calcium-binding protein b. The associations between each CSF synaptic biomarker and hippocampal volume were tested in a linear model in the whole sample. All analyses were adjusted for p-tau, age, sex, *APOE* status and Total Intracranial Volume (TIV). The regression coefficients ( $\beta$ ) and SEs are depicted. All *P* values are corrected for multiple comparisons using the false discovery rate approach.