

# Supplementary Material

## Plasma Glial Fibrillary Acidic Protein Is Associated with <sup>18</sup>F-SMBT-1 PET: Two Putative Astrocyte Reactivity Biomarkers for Alzheimer's Disease

**Supplementary Table 1. Participant characteristics for the subset that underwent <sup>18</sup>F-SMBT-1 imaging within 12 months of blood collection (N=59).** Sex, age, Apolipoprotein E (*APOE*) ε4 allele status, Mini-Mental State Examination (MMSE) scores, plasma glial fibrillary acidic protein (GFAP) levels, plasma Aβ<sub>1-42</sub>/Aβ<sub>1-40</sub> ratios, brain Aβ PET and brain monoamine oxidase B expression assessed using SMBT-1 PET in the supramarginal gyrus (SG), posterior cingulate (PC), lateral temporal (LT), lateral occipital (LO) and superior parietal (SP) are presented. HC, healthy control; MCI, mild cognitively impaired; AD, Alzheimer's disease; PET, positron emission tomography, SMBT-1, (S)-(2-methylpyrid-5-yl)-6-[(3-<sup>18</sup>F-fluoro-2-hydroxy)propoxy] quinoline; SUVR, standardised uptake value ratio. Plasma GFAP levels and SMBT-1 PET SG, PC, LT, LO, and SP SUVRs were higher in Aβ<sup>+</sup> participants (n=15) compared with Aβ<sup>-</sup> participants (n=44) after adjusting for age and sex (p≤0.01).

|   | HC Aβ <sup>-</sup>    | HC Aβ <sup>+</sup>    | MCI Aβ <sup>-</sup>   | MCI Aβ <sup>+</sup>   | AD Aβ <sup>-</sup> | AD Aβ <sup>+</sup>    |
|---|-----------------------|-----------------------|-----------------------|-----------------------|--------------------|-----------------------|
| <b>N (M/F)</b>  | 35 (14/21)            | 11 (6/5)              | 8 (5/3)               | 2 (2/0)               | 1 (1/0)            | 2 (1/1)               |
| <b>Age y, mean±SD (range)</b>                                     | 76.36±5.17<br>(63-86) | 78.61±5.96<br>(71-89) | 71.83±6.37<br>(58-80) | 76.65±0.92<br>(76-77) | 70.00              | 79.35±5.30<br>(76-83) |
| <b><i>APOE</i> ε4 carrier status (N (%))</b>                      | 11 (31.43)            | 7 (63.63)             | 2 (25)                | 0 (0)                 | 0 (0)              | 1 (50)                |
| <b>MMSE (mean±SD)</b>   | 28.54±1.58            | 28.36±1.63            | 28.25±1.67            | 26.00±0.00            | 27.00              | 23.00±8.49            |
| <b>CDR (mean±SD)</b>  | 0.04±0.14             | 0.00±0.00             | 0.31±0.26             | 0.50±0.00             | 0.50               | 0.75±0.35             |
| <b>Plasma GFAP (pg/mL, mean±SD)</b>                               | 122.02±46.34          | 181.33±58.72          | 97.91±26.19           | 140.05±106.25         | 75.44              | 272.33±247.69         |
| <b>Plasma Aβ<sub>1-42</sub>/Aβ<sub>1-40</sub> ratio (mean±SD)</b> | 0.061±0.013           | 0.054±0.010           | 0.061±0.012           | 0.061±0.007           | 0.052              | 0.037±0.013           |
| <b>Aβ PET (centiloid, mean±SD)</b>                                | 0.10±8.11             | 49.15±33.05           | 1.55±6.47             | 40.90±29.42           | -3.80              | 87.65±21.00           |
| <b>SMBT-1 PET SG (SUVR, mean±SD)</b>                              | 1.16±0.10             | 1.29±0.12             | 1.19±0.07             | 1.31±0.08             | 1.07               | 1.29±0.03             |
| <b>SMBT-1 PET PC (SUVR, mean±SD)</b>                              | 1.23±0.12             | 1.34±0.17             | 1.26±0.04             | 1.36±0.22             | 1.18               | 1.40±0.08             |
| <b>SMBT-1 PET LT (SUVR, mean±SD)</b>                              | 1.22±0.13             | 1.35±0.11             | 1.24±0.07             | 1.30±0.01             | 1.16               | 1.36±0.13             |
| <b>SMBT-1 PET LO (SUVR, mean±SD)</b>                              | 0.96±0.10             | 1.06±0.11             | 0.95±0.04             | 1.06±0.06             | 0.89               | 1.20±0.13             |
| <b>SMBT-1 PET SP (SUVR, mean±SD)</b>                              | 0.95±0.11             | 1.04±0.13             | 0.98±0.12             | 1.11±0.09             | 0.93               | 1.14±0.06             |

**Supplementary Table 2. Association of plasma GFAP with <sup>18</sup>F-SMBT-1 PET in participants that underwent <sup>18</sup>F-SMBT-1 imaging within 12 months of blood collection (N=59).** Linear regressions were used to perform the analyses. Age, sex, *APOE* ε4 carrier status, insoluble Aβ (Aβ PET) and soluble Aβ (plasma Aβ<sub>1-42</sub>/Aβ<sub>1-40</sub>) were also added to the model as covariates. Natural log plasma GFAP values were used in the model to satisfy Shapiro-Wilk test of normality of the model residuals. ‘β’ represents the standardized coefficients and ‘p’ represents significance, with *p*<0.05 considered significant (in bold). GFAP, glial fibrillary acidic protein; PET, positron emission tomography; SMBT-1, (S)-(2-methylpyrid-5-yl)-6-[(3-<sup>18</sup>F-fluoro-2-hydroxy)propoxy] quinoline.

|  | <b>Regional<br/>SMBT-1 PET<br/>β (p)</b> | <b>Aβ PET<br/>β (p)</b> | <b>Plasma Aβ<sub>1-42</sub>/Aβ<sub>1-40</sub><br/>β (p)</b> | <b>Age<br/>β (p)</b>   | <b>Sex<br/>β (p)</b>    | <b><i>APOE</i> ε4<br/>carrier status<br/>β (p)</b> |
|--|--|-------------------------|---|------------------------|-------------------------|--|
| <b>A. No covariates included</b>   |  |                         |   |                        |                         |  |
| Supramarginal gyrus  | 0.317 ( <b>0.014</b> )                   | -                       | -   | -                      | -                       | -  |
| Posterior cingulate  | 0.276 ( <b>0.034</b> )                   | -                       | -   | -                      | -                       | -  |
| Lateral temporal   | 0.306 ( <b>0.018</b> )                   | -                       | -   | -                      | -                       | -  |
| Lateral occipital  | 0.224 (0.088)                            | -                       | -   | -                      | -                       | -  |
| Superior parietal  | 0.210 (0.110)                            | -                       | -   | -                      | -                       | -  |
| <b>B. Inclusion of age, sex, and <i>APOE</i> ε4 carrier status</b>                           |  |                         |   |                        |                         |  |
| Supramarginal gyrus  | 0.314 ( <b>0.009</b> )                   | -                       | -   | 0.280 ( <b>0.015</b> ) | 0.403 (< <b>0.001</b> ) | 0.053 (0.647)                                      |
| Posterior cingulate  | 0.268 ( <b>0.026</b> )                   | -                       | -   | 0.293 ( <b>0.012</b> ) | 0.390 ( <b>0.002</b> )  | 0.063 (0.596)                                      |
| Lateral temporal   | 0.258 ( <b>0.030</b> )                   | -                       | -   | 0.300 ( <b>0.011</b> ) | 0.360 ( <b>0.003</b> )  | 0.064 (0.591)                                      |
| Lateral occipital  | 0.201 (0.117)                            | -                       | -   | 0.270 ( <b>0.029</b> ) | 0.392 ( <b>0.002</b> )  | 0.078 (0.523)                                      |
| Superior parietal  | 0.235 ( <b>0.049</b> )                   | -                       | -   | 0.296 ( <b>0.012</b> ) | 0.394 ( <b>0.002</b> )  | 0.097 (0.410)                                      |
| <b>C. Inclusion of insoluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>             |  |                         |   |                        |                         |  |
| Supramarginal gyrus  | 0.166 (0.171)                            | 0.376 ( <b>0.005</b> )  | -   | 0.177 (0.114)          | 0.439 (< <b>0.001</b> ) | -0.025 (0.822)                                     |
| Posterior cingulate  | 0.155 (0.177)                            | 0.401 ( <b>0.002</b> )  | -   | 0.174 (0.120)          | 0.439 (< <b>0.001</b> ) | -0.031 (0.786)                                     |
| Lateral temporal   | 0.134 (0.245)                            | 0.402 ( <b>0.002</b> )  | -   | 0.179 (0.112)          | 0.422 (< <b>0.001</b> ) | -0.027 (0.812)                                     |
| Lateral occipital  | 0.023 (0.859)                            | 0.443 ( <b>0.001</b> )  | -   | 0.173 (0.133)          | 0.425 (< <b>0.001</b> ) | -0.013 (0.909)                                     |
| Superior parietal  | 0.103 (0.375)                            | 0.413 ( <b>0.002</b> )  | -   | 0.176 (0.120)          | 0.437 (< <b>0.001</b> ) | -0.011 (0.924)                                     |
| <b>D. Inclusion of soluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>               |  |                         |   |                        |                         |  |
| Supramarginal gyrus  | 0.300 ( <b>0.009</b> )                   | -                       | -0.252 ( <b>0.028</b> )                                     | 0.234 ( <b>0.036</b> ) | 0.421 (< <b>0.001</b> ) | -0.001 (0.996)                                     |
| Posterior cingulate  | 0.251 ( <b>0.030</b> )                   | -                       | -0.252 ( <b>0.030</b> )                                     | 0.247 ( <b>0.030</b> ) | 0.408 (< <b>0.001</b> ) | 0.009 (0.937)                                      |
| Lateral temporal   | 0.253 ( <b>0.027</b> )                   | -                       | -0.264 ( <b>0.023</b> )                                     | 0.250 ( <b>0.028</b> ) | 0.382 ( <b>0.001</b> )  | 0.005 (0.964)                                      |
| Lateral occipital  | 0.206 (0.094)                            | -                       | -0.273 ( <b>0.022</b> )                                     | 0.216 (0.071)          | 0.416 (< <b>0.001</b> ) | 0.015 (0.902)                                      |
| Superior parietal  | 0.248 ( <b>0.031</b> )                   | -                       | -0.281 ( <b>0.016</b> )                                     | 0.241 ( <b>0.035</b> ) | 0.420 (< <b>0.001</b> ) | 0.032 (0.783)                                      |
| <b>E. Inclusion of insoluble Aβ, soluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b> |  |                         |   |                        |                         |  |
| Supramarginal gyrus  | 0.182 (0.131)                            | 0.310 ( <b>0.026</b> )  | -0.164 (0.155)  | 0.166 (0.136)          | 0.445 (< <b>0.001</b> ) | -0.047 (0.678)                                     |

|                     |               |                        |                |               |                         |                |
|---------------------|---------------|------------------------|----------------|---------------|-------------------------|----------------|
| Posterior cingulate | 0.160 (0.159) | 0.344 ( <b>0.010</b> ) | -0.154 (0.182) | 0.163 (0.143) | 0.443 (< <b>0.001</b> ) | -0.050 (0.658) |
| Lateral temporal    | 0.150 (0.190) | 0.338 ( <b>0.013</b> ) | -0.163 (0.160) | 0.167 (0.134) | 0.425 (< <b>0.001</b> ) | -0.049 (0.667) |
| Lateral occipital   | 0.053 (0.678) | 0.374 ( <b>0.010</b> ) | -0.156 (0.189) | 0.158 (0.170) | 0.434 (< <b>0.001</b> ) | -0.035 (0.760) |
| Superior parietal   | 0.134 (0.251) | 0.339 ( <b>0.014</b> ) | -0.172 (0.145) | 0.164 (0.145) | 0.445 (< <b>0.001</b> ) | -0.032 (0.779) |

**Supplementary Table 3. Association of plasma GFAP with <sup>18</sup>F-SMBT-1 PET.** Linear regressions were used to perform analyses A) in study participants within the AD continuum (HC Aβ+, MCI Aβ+, AD Aβ+; N=21) and B) in cognitively impaired study participants (MCI and AD, N=17). Age, sex, *APOE* ε4 carrier status, soluble Aβ (plasma Aβ<sub>1-42</sub>/Aβ<sub>1-40</sub> ratio), and insoluble Aβ (Aβ PET) were also added to the model as covariates. Natural log plasma GFAP values were used in the model to satisfy Shapiro-Wilk test of normality of the model residuals. ‘β’ represents the standardized coefficients and ‘p’ represents significance, with *p*<0.05 considered significant (in bold). GFAP, glial fibrillary acidic protein; PET, positron emission tomography; <sup>18</sup>F-SMBT-1, (S)-(2-methylpyrid-5-yl)-6-[(3-<sup>18</sup>F-fluoro-2-hydroxy)propoxy] quinoline.

| A  | Regional SMBT-1 PET<br>β (p) | Plasma Aβ <sub>1-42</sub> /Aβ <sub>1-40</sub><br>β (p) | Aβ PET<br>β (p) | Age<br>β (p)  | Sex<br>β (p)           | <i>APOE</i> ε4 carrier status<br>β (p) |
|--|------------------------------|--|-----------------|---------------|------------------------|--|
| <b>A. No covariates included</b>   |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.153 (0.508)                | -  | -               | -             | -                      | -                                      |
| <b>B. Inclusion of age, sex, and <i>APOE</i> ε4 carrier status</b>                           |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.293 (0.157)                | -  | -               | 0.129 (0.487) | 0.719 (0.002)          | -0.151 (0.468)                         |
| <b>C. Inclusion of soluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>               |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.233 (0.291)                | -0.172 (0.432)   | -               | 0.152 (0.426) | 0.641 ( <b>0.010</b> ) | -0.118 (0.581)                         |
| <b>D. Inclusion of insoluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>             |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.293 (0.150)                | -  | 0.253 (0.217)   | 0.077 (0.679) | 0.686 ( <b>0.003</b> ) | -0.241 (0.271)                         |
| <b>E. Inclusion of soluble Aβ, insoluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b> |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.274 (0.227)                | -0.055 (0.826)   | 0.228 (0.344)   | 0.090 (0.656) | 0.665 ( <b>0.009</b> ) | -0.222 (0.361)                         |

| B  | Regional SMBT-1 PET<br>β (p) | Plasma Aβ <sub>1-42</sub> /Aβ <sub>1-40</sub><br>β (p) | Aβ PET<br>β (p) | Age<br>β (p)  | Sex<br>β (p)           | <i>APOE</i> ε4 carrier status<br>β (p) |
|--|------------------------------|--|-----------------|---------------|------------------------|--|
| <b>A. No covariates included</b>   |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.664 ( <b>0.004</b> )       | -  | -               | -             | -                      | -                                      |
| <b>B. Inclusion of age, sex, and <i>APOE</i> ε4 carrier status</b>                           |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.630 ( <b>0.003</b> )       | -  | -               | 0.207 (0.258) | 0.497 ( <b>0.009</b> ) | -0.073 (0.689)                         |
| <b>C. Inclusion of soluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>               |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.567 ( <b>0.009</b> )       | -0.195 (0.285)   | -               | 0.211 (0.246) | 0.456 ( <b>0.018</b> ) | -0.095 (0.603)                         |
| <b>D. Inclusion of insoluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b>             |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.496 (0.065)                | -  | 0.245 (0.441)   | 0.176 (0.350) | 0.454 ( <b>0.023</b> ) | -0.168 (0.454)                         |
| <b>E. Inclusion of soluble Aβ, insoluble Aβ, age, sex, and <i>APOE</i> ε4 carrier status</b> |                              |  |                 |               |                        |  |
| Supramarginal gyrus  | 0.495 (0.071)                | -0.166 (0.404)   | 0.150 (0.658)   | 0.192 (0.321) | 0.436 ( <b>0.032</b> ) | -0.150 (0.511)                         |

**Supplementary Table 4. Association of A $\beta$  PET with plasma GFAP and <sup>18</sup>F-SMBT-1 PET in participants that underwent <sup>18</sup>F-SMBT-1 imaging within 12 months of blood collection.** Linear regressions were used to perform the analyses in study participants (N=55) after removing outliers and utilizing natural log plasma GFAP values in the model to satisfy Shapiro-Wilk test of normality of model residuals. Soluble A $\beta$ , age, sex, and *APOE*  $\epsilon$ 4 carrier status were also added to the model as covariates. Participants with standardized model residuals larger than an absolute value of  $\pm 2$  were considered as outliers. ' $\beta$ ' represents the standardized coefficients and ' $p$ ' represents significance, with  $p < 0.05$  considered significant (in bold). GFAP, glial fibrillary acidic protein; SMBT-1, (S)-(2-methylpyrid-5-yl)-6-[(3-<sup>18</sup>F-fluoro-2-hydroxy)propoxy] quinoline; PET, positron emission tomography.

| <b>Plasma GFAP<br/><math>\beta</math> (<math>p</math>)</b> | <b>Regional SMBT-1 PET<br/><math>\beta</math> (<math>p</math>)</b> | <b>Plasma A<math>\beta_{1-42}</math>/A<math>\beta_{1-40}</math><br/><math>\beta</math> (<math>p</math>)</b> | <b>Age<br/><math>\beta</math> (<math>p</math>)</b> | <b>Sex<br/><math>\beta</math> (<math>p</math>)</b> | <b><i>APOE</i> <math>\epsilon</math>4<br/>carrier status<br/><math>\beta</math> (<math>p</math>)</b> |
|--|--|---|--|--|--|
| 0.393 ( <b>0.002</b> )                                     | 0.385 (< <b>0.001</b> ) <i>Supramarginal gyrus</i>                 | -0.276 ( <b>0.009</b> )   | -0.001 (0.990)                                     | -0.222 (0.050)                                     | 0.057 (0.579)  |
| 0.438 (< <b>0.001</b> )                                    | 0.306 ( <b>0.007</b> ) <i>Posterior cingulate</i>                  | -0.263 ( <b>0.017</b> )   | -0.005 (0.962)                                     | -0.255 ( <b>0.031</b> )                            | 0.068 (0.527)  |
| 0.458 (< <b>0.001</b> )                                    | 0.286 ( <b>0.011</b> ) <i>Lateral temporal</i>                     | -0.279 ( <b>0.013</b> )   | 0.009 (0.934)                                      | -0.298 ( <b>0.011</b> )                            | 0.059 (0.594)  |
| 0.435 (< <b>0.001</b> )                                    | 0.355 ( <b>0.002</b> ) <i>Lateral occipital</i>                    | -0.302 ( <b>0.006</b> )   | -0.054 (0.600)                                     | -0.236 ( <b>0.041</b> )                            | 0.062 (0.554)  |
| 0.419 ( <b>0.001</b> )                                     | 0.333 ( <b>0.003</b> ) <i>Superior parietal</i>                    | -0.298 ( <b>0.007</b> )   | -0.010 (0.921)                                     | -0.228 (0.053)                                     | 0.103 (0.326)  |