**Supplementary Material**

**Validation of Plasma Proteomic Biomarkers Relating to Brain Amyloid Burden in the EMIF-Alzheimer’s Disease Multimodal Biomarker Discovery Cohort**

**Supplementary Table 1.** Logistic regression (age as covariate) results for each protein with amyloid status as the outcome variable in AD subjects only. \*statistically significant <0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Logistic Regression** | | |
| **Sample set** | **protein** | **beta** | ***p*** | ***q*** | |
| Oxford | FCN2 | 0.722 | 0.062 | 0.635 | |
| FGG | 0.140 | 0.620 | 0.946 | |
| Cystatin C | -0.307 | 0.218 | 0.803 | |
| Clusterin | -0.468 | 0.083 | 0.635 | |
| B2M | -0.484 | 0.035\* | 0.635 | |
| AGP | 0.118 | 0.665 | 0.946 | |
| CP | 0.043 | 0.868 | 0.956 | |
| A2M | -0.261 | 0.288 | 0.803 | |
| ApoA1 | 0.060 | 0.817 | 0.956 | |
| ApoC3 | -0.247 | 0.309 | 0.803 | |
| apoE | -0.353 | 0.131 | 0.675 | |
| TTR | -0.123 | 0.630 | 0.946 | |
| CFH | -0.394 | 0.102 | 0.635 | |
| CRP | -0.052 | 0.849 | 0.956 | |
| A1AT | 0.227 | 0.540 | 0.946 | |
| PEDF | 0.033 | 0.907 | 0.956 | |
| SAP | -0.264 | 0.241 | 0.803 | |
| CC4 | 0.658 | 0.050\* | 0.635 | |
| BDNF | -0.107 | 0.671 | 0.946 | |
| Cathepsin D | -0.232 | 0.311 | 0.803 | |
| sICAM-1 | 0.038 | 0.881 | 0.956 | |
| RANTES | 0.014 | 0.954 | 0.956 | |
| NCAM | 0.218 | 0.452 | 0.877 | |
| sVCAM-1 | 0.029 | 0.911 | 0.956 | |
| PAI.1 | -0.199 | 0.381 | 0.817 | |
| Cardiff | CR1 | 0.023 | 0.952 | 0.956 | |
| TCC | -0.312 | 0.234 | 0.803 | |
| CFB | 0.203 | 0.650 | 0.946 | |
| CFI | 0.024 | 0.956 | 0.956 | |
| Eotaxin | -0.282 | 0.338 | 0.805 | |
| MCP | -0.249 | 0.395 | 0.817 | |

**Supplementary Table 2.** Logistic regression (age as covariate) results for each protein with amyloid status as the outcome variable in MCI subjects only. \*statistically significant <0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Logistic Regression** | | |
| **Sample set** | **protein** | **beta** | ***p*** | ***q*** |
| Oxford | FCN2 | 0.241 | 0.046\* | 0.264 |
| FGG | 0.246 | 0.037\* | 0.264 |
| Cystatin C | -0.074 | 0.499 | 0.736 |
| Clusterin | 0.065 | 0.550 | 0.875 |
| B2M | -0.213 | 0.060 | 0.264 |
| AGP | 0.093 | 0.403 | 0.658 |
| CP | 0.170 | 0.127 | 0.342 |
| A2M | 0.000 | 0.998 | 0.998 |
| ApoA1 | 0.163 | 0.148 | 0.343 |
| ApoC3 | 0.048 | 0.663 | 0.859 |
| apoE | -0.305 | 0.006\* | 0.188 |
| TTR | 0.001 | 0.991 | 0.998 |
| CFH | 0.035 | 0.748 | 0.859 |
| CRP | -0.158 | 0.155 | 0.343 |
| A1AT | 0.009 | 0.935 | 0.998 |
| PEDF | -0.110 | 0.321 | 0.562 |
| SAP | -0.087 | 0.425 | 0.658 |
| CC4 | 0.217 | 0.053 | 0.264 |
| BDNF | 0.021 | 0.846 | 0.936 |
| Cathepsin D | -0.170 | 0.118 | 0.342 |
| sICAM-1 | -0.106 | 0.327 | 0.562 |
| RANTES | 0.036 | 0.742 | 0.859 |
| NCAM | 0.177 | 0.121 | 0.342 |
| sVCAM-1 | -0.038 | 0.728 | 0.859 |
| PAI.1 | -0.127 | 0.235 | 0.454 |
| Cardiff | CR1 | 0.234 | 0.031\* | 0.264 |
| TCC | 0.176 | 0.132 | 0.342 |
| CFB | -0.137 | 0.189 | 0.391 |
| CFI | -0.263 | 0.018\* | 0.264 |
| Eotaxin | 0.198 | 0.101 | 0.342 |
| MCP | 0.037 | 0.725 | 0.859 |

**Supplementary Table 3.** Logistic regression (age as covariate) results for each protein with amyloid status as the outcome variable in cognitively healthy control subjects only. \*statistically significant <0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Logistic Regression** | | |
| **Sample set** | **protein** | **beta** | ***p*** | ***q*** |
| Oxford | FCN2 | 0.305 | 0.010\* | 0.180 |
| FGG | -0.137 | 0.262 | 0.427 |
| Cystatin C | -0.228 | 0.066 | 0.256 |
| Clusterin | -0.166 | 0.172 | 0.368 |
| B2M | -0.263 | 0.053 | 0.256 |
| AGP | -0.216 | 0.087 | 0.298 |
| CP | -0.254 | 0.047\* | 0.256 |
| A2M | -0.162 | 0.202 | 0.368 |
| ApoA1 | -0.245 | 0.060 | 0.256 |
| ApoC3 | -0.016 | 0.891 | 0.920 |
| apoE | -0.175 | 0.160 | 0.368 |
| TTR | -0.180 | 0.145 | 0.368 |
| CFH | -0.178 | 0.146 | 0.368 |
| CRP | 0.011 | 0.924 | 0.924 |
| A1AT | -0.359 | 0.014\* | 0.180 |
| PEDF | -0.020 | 0.872 | 0.920 |
| SAP | 0.139 | 0.235 | 0.404 |
| CC4 | 0.150 | 0.201 | 0.368 |
| BDNF | 0.094 | 0.427 | 0.614 |
| Cathepsin D | -0.097 | 0.436 | 0.614 |
| sICAM-1 | -0.288 | 0.045\* | 0.256 |
| RANTES | 0.084 | 0.471 | 0.634 |
| NCAM | -0.017 | 0.888 | 0.920 |
| sVCAM-1 | -0.017 | 0.884 | 0.920 |
| PAI.1 | 0.156 | 0.180 | 0.368 |
| Cardiff | CR1 | -0.667 | 0.017\* | 0.180 |
| TCC | -0.044 | 0.781 | 0.920 |
| CFB | 0.092 | 0.497 | 0.642 |
| CFI | -0.242 | 0.137 | 0.368 |
| Eotaxin | 0.143 | 0.276 | 0.428 |
| MCP | 0.040 | 0.767 | 0.920 |

**Supplementary Table 4.** Logistic regression (age as covariate) results for each protein with amyloid status as the outcome variable in *APOE ε4* carrier subjects only. \*statistically significant <0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Logistic Regression** | | |
| **Sample set** | **protein** | **beta** | ***p*** | ***q*** |
| Oxford | FCN2 | 0.628 | 0.000\* | 0.000\* |
| FGG | -0.258 | 0.013\* | 0.120 |
| Cystatin C | -0.127 | 0.234 | 0.409 |
| Clusterin | -0.136 | 0.204 | 0.400 |
| B2M | -0.111 | 0.305 | 0.431 |
| AGP | 0.033 | 0.761 | 0.795 |
| CP | 0.031 | 0.770 | 0.795 |
| A2M | 0.064 | 0.575 | 0.660 |
| ApoA1 | -0.071 | 0.503 | 0.606 |
| ApoC3 | -0.074 | 0.480 | 0.606 |
| apoE | -0.108 | 0.306 | 0.431 |
| TTR | -0.136 | 0.200 | 0.400 |
| CFH | -0.023 | 0.831 | 0.831 |
| CRP | -0.236 | 0.025\* | 0.129 |
| A1AT | -0.221 | 0.050\* | 0.192 |
| PEDF | -0.121 | 0.251 | 0.409 |
| SAP | -0.187 | 0.081 | 0.280 |
| CC4 | 0.405 | 0.001\* | 0.009\* |
| BDNF | -0.168 | 0.106 | 0.329 |
| Cathepsin D | -0.253 | 0.017\* | 0.120 |
| sICAM-1 | -0.141 | 0.179 | 0.400 |
| RANTES | -0.241 | 0.019\* | 0.120 |
| NCAM | 0.131 | 0.244 | 0.409 |
| sVCAM-1 | 0.071 | 0.509 | 0.606 |
| PAI.1 | -0.160 | 0.122 | 0.345 |
| Cardiff | CR1 | 0.047 | 0.707 | 0.783 |
| TCC | 0.134 | 0.297 | 0.431 |
| CFB | 0.090 | 0.461 | 0.606 |
| CFI | -0.151 | 0.206 | 0.400 |
| Eotaxin | 0.325 | 0.030\* | 0.133 |
| MCP | 0.202 | 0.207 | 0.400 |

**Supplementary Table 5.** Logistic regression (age as covariate) results for each protein with amyloid status as the outcome variable in *APOE* *ε4* non-carrier subjects only. \*statistically significant <0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Logistic Regression** | | |
| **Sample set** | **protein** | **beta** | ***p*** | ***q*** |
| Oxford | FCN2 | 0.395 | 0.000\* | 0.005\* |
| FGG | 0.083 | 0.406 | 0.716 |
| Cystatin C | -0.206 | 0.043\* | 0.222 |
| Clusterin | -0.241 | 0.017\* | 0.125 |
| B2M | -0.427 | 0.000\* | 0.005\* |
| AGP | -0.062 | 0.545 | 0.769 |
| CP | -0.021 | 0.836 | 0.894 |
| A2M | -0.033 | 0.751 | 0.894 |
| ApoA1 | -0.052 | 0.609 | 0.821 |
| ApoC3 | 0.077 | 0.439 | 0.716 |
| apoE | -0.022 | 0.827 | 0.894 |
| TTR | 0.062 | 0.539 | 0.769 |
| CFH | 0.012 | 0.903 | 0.908 |
| CRP | 0.082 | 0.416 | 0.713 |
| A1AT | -0.190 | 0.081 | 0.288 |
| PEDF | 0.110 | 0.281 | 0.669 |
| SAP | 0.065 | 0.513 | 0.769 |
| CC4 | 0.175 | 0.075 | 0.288 |
| BDNF | 0.043 | 0.668 | 0.863 |
| Cathepsin D | -0.249 | 0.020\* | 0.125 |
| sICAM-1 | -0.177 | 0.084 | 0.288 |
| RANTES | 0.037 | 0.713 | 0.884 |
| NCAM | -0.025 | 0.799 | 0.894 |
| sVCAM-1 | -0.161 | 0.112 | 0.348 |
| PAI.1 | -0.090 | 0.384 | 0.716 |
| Cardiff | CR1 | -0.165 | 0.268 | 0.669 |
| TCC | 0.090 | 0.387 | 0.716 |
| CFB | -0.087 | 0.417 | 0.716 |
| CFI | -0.362 | 0.002\* | 0.022\* |
| Eotaxin | 0.125 | 0.234 | 0.660 |
| MCP | -0.012 | 0.908 | 0.908 |

**Supplementary Table 6.** AUC statistics per protein, for the classification of normal / abnormal brain amyloid status, within *APOE* ε4 carrier/non-carrier groups separately.

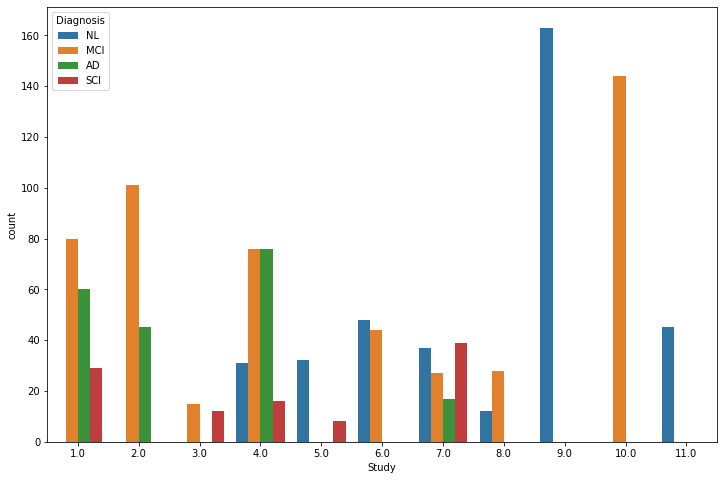
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **APOE ε4 status** | **Optimal cutpoint** | **Sensitivity** | **Specificity** | **AUC** | **CI.low** | **CI.up** |
| A1AT | carrier | 142278.725 | 0.408 | 0.706 | 0.563 | 0.483 | 0.650 |
| A1AT | non-carrier | 273159.570 | 0.544 | 0.571 | 0.546 | 0.468 | 0.623 |
| apoE | carrier | 93.568 | 0.624 | 0.456 | 0.527 | 0.455 | 0.609 |
| apoE | non-carrier | 138.613 | 0.836 | 0.233 | 0.501 | 0.426 | 0.570 |
| B2M | carrier | 2957.260 | 0.276 | 0.872 | 0.554 | 0.478 | 0.632 |
| B2M | non-carrier | 6191.573 | 0.658 | 0.524 | 0.604 | 0.531 | 0.676 |
| Cathepsin D | carrier | 270.670 | 0.395 | 0.805 | 0.597 | 0.526 | 0.674 |
| Cathepsin D | non-carrier | 393.785 | 0.784 | 0.363 | 0.573 | 0.496 | 0.642 |
| CC4 | carrier | 42726.128 | 0.752 | 0.450 | 0.621 | 0.545 | 0.698 |
| CC4 | non-carrier | 73547.781 | 0.467 | 0.693 | 0.556 | 0.488 | 0.627 |
| FCN2 | carrier | 18215589.820 | 0.630 | 0.682 | 0.674 | 0.596 | 0.745 |
| FCN2 | non-carrier | 24620022.060 | 0.515 | 0.772 | 0.626 | 0.547 | 0.696 |
| CFI | carrier | 25047.264 | 0.460 | 0.684 | 0.552 | 0.469 | 0.634 |
| CFI | non-carrier | 26770.591 | 0.529 | 0.647 | 0.596 | 0.524 | 0.673 |
| Age | carrier | 65.010 | 0.731 | 0.534 | 0.652 | 0.585 | 0.714 |
| Age | non-carrier | 67.355 | 0.708 | 0.532 | 0.643 | 0.580 | 0.706 |

**Supplementary Table 7.** AUC statistics per protein, for the classification of normal / abnormal brain amyloid status, within diagnostic groups separately.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Diagnosis** | **Optimal cutpoint** | **Sensitivity** | **Specificity** | **AUC** | **CI.low** | **CI.up** |
| A1AT | MCI | 253270.314 | 0.481 | 0.627 | 0.526 | 0.442 | 0.612 |
| A1AT | AD | 54461.556 | 0.299 | 0.917 | 0.500 | 0.250 | 0.730 |
| A1AT | CN | 3758206.009 | 0.925 | 0.240 | 0.559 | 0.466 | 0.655 |
| apoE | MCI | 115.549 | 0.722 | 0.449 | 0.583 | 0.501 | 0.669 |
| apoE | AD | 111.074 | 0.720 | 0.563 | 0.626 | 0.396 | 0.864 |
| apoE | CN | 104.386 | 0.693 | 0.431 | 0.545 | 0.462 | 0.632 |
| B2M | MCI | 18721.188 | 0.948 | 0.150 | 0.535 | 0.448 | 0.623 |
| B2M | AD | 7032.937 | 0.669 | 0.643 | 0.671 | 0.421 | 0.899 |
| B2M | CN | 7295.702 | 0.753 | 0.419 | 0.589 | 0.497 | 0.679 |
| Cathepsin D | MCI | 452.920 | 0.859 | 0.261 | 0.551 | 0.470 | 0.629 |
| Cathepsin D | AD | 319.070 | 0.600 | 0.611 | 0.602 | 0.418 | 0.778 |
| Cathepsin D | CN | 272.795 | 0.396 | 0.741 | 0.561 | 0.464 | 0.655 |
| CC4 | MCI | 44884.347 | 0.764 | 0.387 | 0.567 | 0.484 | 0.643 |
| CC4 | AD | 77450.398 | 0.401 | 0.882 | 0.655 | 0.453 | 0.865 |
| CC4 | CN | 78252.733 | 0.354 | 0.777 | 0.551 | 0.459 | 0.642 |
| FCN2 | MCI | 22524455.125 | 0.500 | 0.712 | 0.597 | 0.514 | 0.681 |
| FCN2 | AD | 18939335.605 | 0.723 | 0.643 | 0.679 | 0.446 | 0.908 |
| FCN2 | CN | 30220128.285 | 0.276 | 0.891 | 0.571 | 0.479 | 0.657 |
| CFI | MCI | 26825.973 | 0.462 | 0.713 | 0.587 | 0.511 | 0.661 |
| CFI | AD | 29249.109 | 0.875 | 0.333 | 0.508 | 0.033 | 0.929 |
| CFI | CN | 24171.476 | 0.424 | 0.752 | 0.566 | 0.459 | 0.678 |
| Age | MCI | 67.350 | 0.709 | 0.483 | 0.603 | 0.536 | 0.665 |
| Age | AD | 68.900 | 0.571 | 0.200 | 0.381 | 0.206 | 0.555 |
| Age | CN | 66.100 | 0.593 | 0.582 | 0.570 | 0.484 | 0.655 |

**Supplementary Table 8.** Ethical approval committee of each center

|  |  |  |  |
| --- | --- | --- | --- |
| Center | Part of multi-center | Country | Approval Committee |
| Aristotle University, Thessaloniki | DESCRIPA, EDAR, Pharmacog | Greece | Aristotle University of Thessaloniki Medical School Ethics Committee |
| Central Institute for Mental Health, Mannheim | EDAR | Germany | Ethics Committee of the Medical Faculty Mannheim, University of Heidelberg |
| GAP, San Sebastian | - | Spain | Ethic and Clinical Research Committee Donostia |
| Hôpital Timone Adultes, Marseille | Pharmacog | France | Ethics committee Inserm and Aix Marseille University |
| Hospital Clínic de Barcelona IDIBAPS | Pharmacog | Spain | The Healthcare Ethics Committee of the Hospital Clínic |
| Hospital de la Santa Creu i Sant Pau, Barcelona | EDAR | Spain | Central Clinical Research and Clinical Trials Unit (UICEC Sant Pau) |
| INSERM, Toulouse | Pharmacog | France | INSERM Ethical Committee |
| IRCCS-FBF, Brescia | Pharmacog | Italy | Ethic Committee of the IRCCS San Giovanni di Dio FBF |
| IRCCS-SDN, Napels | Pharmacog | Italy | Comitato Etico IRCCS Pascale - Napoli |
| Karolinska Institutet, Stockholm | EDAR | Sweden | Ethics Committee at Karolinska Institutet |
| Katholieke Universiteit, Leuven | EDAR | Belgium | Ethische commissie onderzoek UZ/KU Leuven |
| Lausanne University Hospital, Lausanne | - | Switzerland | Research Ethics Committee Lausanne University Hospital |
| Maastricht University, Maastricht | DESCRIPA, EDAR | Netherlands | Medical ethical committee Maastricht University Medical Center |
| Rigshospitalet, Copenhagen | EDAR | Denmark | Committee on Health Research Ethics, Region of Denmark |
| University of Mediterranean, Marseille | Pharmacog | France | Ethics committee of Mediterranean University |
| University of Lille, Lille | Pharmacog | France | University of Lille Ethics committee |
| University of Leipzig, Leipzig | Pharmacog | Germany | Ethical Committee at the Medical Faculty, Leipzig University |
| University of Essen, Essen | Pharmacog | Germany | Ethical Committee at the Medical Faculty, University Hospital Essen |
| University of Antwerp, Antwerp | - | Belgium | Ethics committee University of Antwerp |
| University of Genoa, Genoa | Pharmacog | Italy | Ethical Committee of University of Genoa |
| University of Gothenburg, Gothenburg | - | Sweden | Ethics Committee, University of Gothenburg |
| University of Perugia, Perugia | Pharmacog | Italy | Human ethics Committee of the University of Perugia |
| VU Medical Center, Amsterdam | EDAR, Pharmacog | Netherlands | Medical ethics committee VU Medical Center |

  
**Supplementary Figure 1. Histogram displaying the difference in the distribution of participants between the ‘Oxford’ and ‘Cardiff’ sample sets, comprised from 11 European cohorts.** AD, Alzheimer’s disease, MCI, mild cognitive impairment, NL, cognitively healthy control, SCI, subjective cognitive impairment.



**Supplementary Figure 2. AUC and corresponding 95% confidence intervals plotted per protein, for the classification of normal/abnormal brain amyloid status.** AUC, area under the curve.