# Ptau-Aβ$_{42}$ Ratio as a Continuous Trait for Biomarker Discovery for Early Stage Alzheimer's Disease in Multiplex Immunoassay Panels of Cerebrospinal Fluid

## *Supplement 1*

**Joint Analyses of Cerebrospinal Fluid (CSF) Fatty Acid Binding Protein (H-FABP) and Visinin-like Protein-1 (VILIP-1) or Cartilage Glycoprotein-39 (YKL-40)**

For the subjects included in the Knight-Alzheimer's Disease Research Center (ADRC) series, the correlation of CSF H-FABP to CSF ptau ($r^2$ = 0.67) is higher than the correlation between CSF ptau and CSF YKL-40 ($r^2$ = 0.56) but lower than the correlation with CSF VILIP-1 ($r^2$ = 0.76). CSF H-FABP shows the same correlation with these two analytes ($r^2$ = 0.63). To further evaluate the significance of the association of CSF H-FABP with CSF ptau-A$\beta_{42}$ ratio we performed multivariate analyses that included these two already identified biomarkers (1; 2). Our analysis showed that CSF YKL-40 was associated with CSF ptau-A$\beta_{42}$ ratio (*p*-value = 3.35E-2) independently of CSF H-FABP (*p*-value = 1.47e-05). Similarly, the joint analysis showed significant association for CSF VILIP-1 and CSF H-FABP (*p*-values = 7.31e-06 and 2.49e-03 respectively). These results demonstrate that CSF H-FABP is adding non-redundant information to the predictive model for CSF ptau-A$\beta_{42}$ ratio.

**Supplemental Methods**

**Alzheimer's Disease Neuroimaging Initiative (ADNI)**

The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public–private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The principal investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California—San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the USA and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 – 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

**Gene Expression**

Expression studies were carried out using from the parietal lobe obtained through the Washington University-ADRC Neuropathology Core. AD changes were measured using Braak and Braak staging (3). All AD cases had a Braak and Braak score of 5 or 6. Among the non-demented individuals 24 brains had a Braak and Braak score ranging from 1–4 indicating the presence of some tangle pathology. Total RNA was extracted from a tissue sample representing the parietal lobe of 82 AD cases and 39 non-demented individuals, using the RNeasy mini kit (Qiagen) following the manufacturer's protocol. cDNA was prepared from total RNA, using the High-Capacity cDNA Archive kit (ABI). Gene expression was analyzed by real-time polymerase chain reaction (PCR), using an ABI-7900 real-time PCR system. TaqMan assay (Life Technologies, Hs00997360_m1) was used to quantify H-FABP total mRNA levels. GAPDH (TaqMan assay, Hs02758991_g1) was used as the reference gene. Each real-time PCR run included within-plate triplicates for each sample. Real-time data were analyzed using the comparative Ct method (4). The Ct values for each sample were normalized with the Ct value for the housekeeping gene, GADPH, and were corrected for the PCR efficiency of each assay, although the efficiency of all reactions was close to 100%. Only samples with a standard error of 0.15 were analyzed.

**Multiple Testing Correction**

We borrowed the ideas of the method termed SimpleM (5), which was developed to account of the linkage disequilibrium while correcting for the number of hypothesis tested in genome-wide association studies. The method employs principal component analysis to determine the minimal number of independent dimensions required to capture the variance observed in the sample. Once this number of independent components is inferred, it employs standard Bonferroni correction to adjust for multiple testing (5). Accordingly, we applied principal component analysis to determine the number of components that capture the 95% of the variance of the panel of analytes, and corrected for that number for each of the studies.
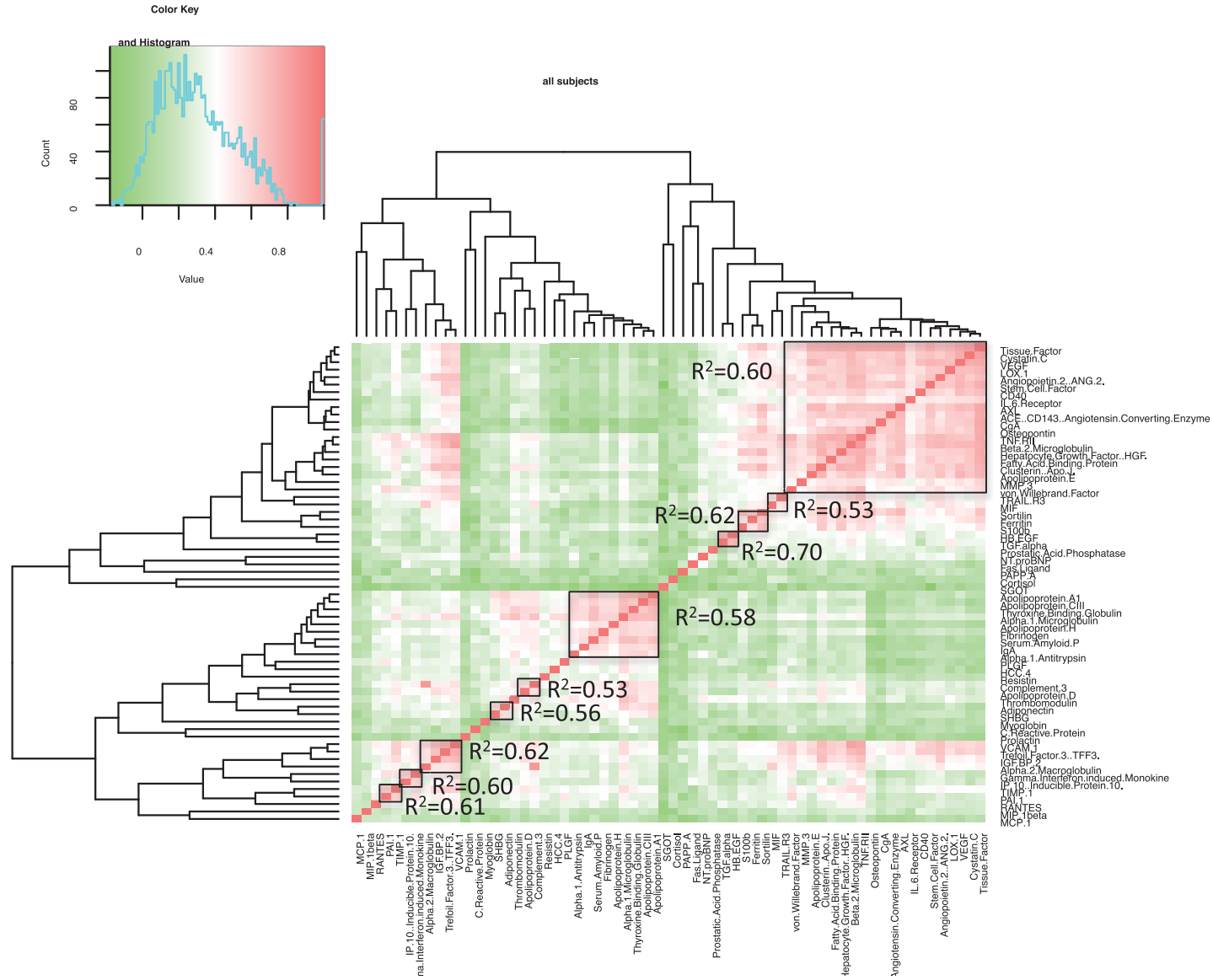
**Figure S1.** Correlation among the 64 analytes that surpass quality control criteria.
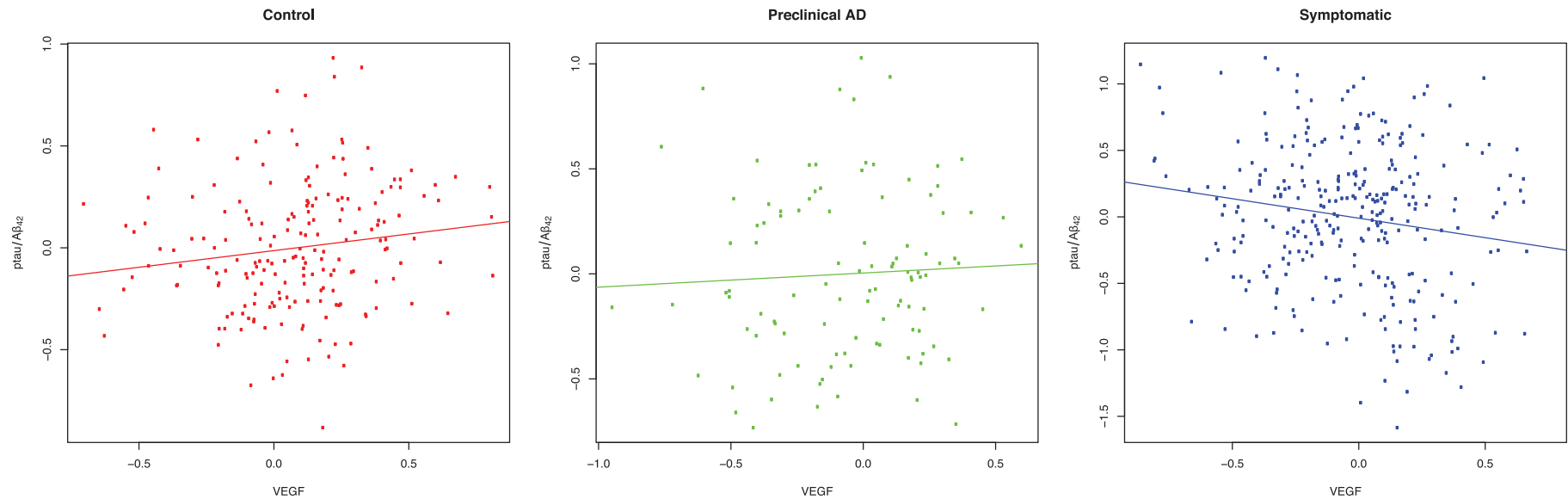
**Figure S2.** Distribution of cerebrospinal fluid (CSF) vascular endothelial growth factor (VEGF) in the Knight-ADRC and the ADNI studies, stratified by clinical status*. VEGF showed a negative effect in the multivariate model but a positive effect in the single analyte analysis. The stratified analysis revealed that the change of direction occurs only in the cases, there is a lack of significant association in the preclinical subjects, and that there is a positive effect in the control stratum. *CSF ptau-A$\beta_{42}$ ratio corrected by age, sex, *APOE* genotypes, CSF fatty acid binding protein, hepatocyte growth factor and macrophage migration inhibitory factor analyte levels. AD, Alzheimer's disease.

**Table S1.** Sample demographics.

|  | Knight-ADRC | ADNI |
|---|---|---|
| *n* | 311 | 293 |
| Age | 72 (±7) | 76 (±6) |
| Male/Female (%) | 39/61 | 60/40 |
| Clinical Dementia Rating |  |  |
| 0: Normal | 217 | 83 |
| 0.5: Very Mild Dementia | 64 | 161 |
| 1: Mild Dementia | 29 | 45 |
| 2: Moderate Dementia | 1 | 3 |
| 3: Severe Dementia | 0 | 1 |
| APOE ε4 (%) | 129 (39%) | 143 (49%) |

ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRC, Alzheimer's Disease Research Center; APOE, apolipoprotein E.

**Table S2.** Correlation matrix for the 64 analytes that met quality control (QC) criterion.

See Supplement 2 (Excel file).

**Table S3.** Association of the 64 analytes that met QC criteria with CSF ptau-A$\beta_{42}$ ratio.

| Analyte | *p*-Value | | | | Effect | |
|---|---|---|---|---|---|---|
| | Analyte | APOE Gen. | Age | Sex | Analyte | R Squared |
| *Knight-ADRC* | | | | | | |
| Fatty.Acid.Binding.Protein | 4.77E-18 | 6.61E-11 | 2.91E-06 | 3.86E-01 | 0.54 | 0.40 |
| TNF.RII | 2.64E-13 | 9.12E-13 | 3.65E-04 | 7.81E-01 | 0.91 | 0.35 |
| Tissue.Factor | 9.18E-13 | 2.69E-14 | 1.48E-09 | 8.63E-01 | 0.54 | 0.35 |
| Hepatocyte.Growth.Factor..HGF. | 4.77E-11 | 1.43E-12 | 1.87E-08 | 8.42E-01 | 0.88 | 0.33 |
| MMP.3 | 2.50E-03 | 2.22E-12 | 2.06E-09 | 5.87E-01 | 0.21 | 0.25 |
| CgA | 1.52E-10 | 2.07E-13 | 1.04E-11 | 3.20E-01 | 1.11 | 0.32 |
| Stem.Cell.Factor | 1.08E-08 | 2.60E-14 | 3.92E-09 | 9.01E-01 | 0.64 | 0.31 |
| Sortilin | 3.22E-08 | 3.04E-12 | 3.72E-10 | 9.84E-01 | 0.89 | 0.30 |
| LOX.1 | 1.54E-07 | 1.39E-13 | 1.18E-10 | 6.17E-01 | 0.51 | 0.29 |
| Beta.2.Microglobulin | 3.36E-07 | 1.02E-12 | 1.01E-07 | 9.49E-01 | 0.70 | 0.29 |
| TRAIL.R3 | 3.67E-07 | 6.36E-11 | 1.23E-05 | 6.49E-01 | 0.82 | 0.29 |
| Angiopoietin.2..ANG.2. | 2.01E-06 | 3.22E-13 | 2.69E-08 | 7.64E-01 | 0.58 | 0.28 |
| NT.proBNP | 1.47E-03 | 3.50E-11 | 2.27E-08 | 6.63E-01 | 0.34 | 0.25 |
| Alpha.2.Macroglobulin | 1.05E-04 | 1.58E-12 | 3.03E-06 | 7.34E-01 | 0.47 | 0.26 |
| HB.EGF | 2.27E-03 | 1.14E-10 | 4.32E-11 | 8.96E-01 | 0.29 | 0.25 |
| Osteopontin | 3.82E-22 | 1.24E-14 | 1.52E-08 | 3.08E-01 | 0.92 | 0.43 |
| Cystatin.C | 3.19E-06 | 2.44E-13 | 2.07E-10 | 9.80E-01 | 0.48 | 0.28 |
| Apolipoprotein.E | 4.83E-05 | 3.45E-15 | 5.92E-09 | 7.31E-01 | 0.45 | 0.27 |
| ACE | 6.18E-05 | 6.93E-13 | 2.56E-10 | 8.28E-01 | 0.39 | 0.27 |
| S100b | 1.56E-04 | 6.76E-11 | 1.96E-09 | 5.61E-01 | 0.60 | 0.26 |
| Clusterin..Apo.J. | 5.11E-05 | 7.78E-12 | 1.58E-08 | 5.76E-01 | 0.55 | 0.27 |
| TGF.alpha | 3.04E-02 | 1.45E-11 | 4.09E-11 | 8.28E-01 | 0.33 | 0.24 |
| von.Willebrand.Factor | 1.31E-04 | 2.60E-12 | 4.60E-07 | 9.77E-01 | 0.43 | 0.26 |
| MIF | 1.80E-09 | 4.56E-11 | 3.03E-06 | 7.23E-01 | 0.74 | 0.31 |
| Ferritin | 2.33E-04 | 9.22E-12 | 1.04E-08 | 5.63E-01 | 0.45 | 0.26 |
| AXL | 3.16E-08 | 7.23E-13 | 6.92E-11 | 8.79E-01 | 0.53 | 0.30 |
| IGF.BP.2 | 1.99E-05 | 6.44E-12 | 3.23E-06 | 4.43E-01 | 0.89 | 0.27 |
| Adiponectin | 4.77E-01 | 1.05E-11 | 4.81E-10 | 9.06E-01 | 0.04 | 0.23 |
| Cortisol | 4.81E-01 | 5.58E-12 | 2.15E-10 | 9.11E-01 | 0.08 | 0.23 |
| IL.6.Receptor | 4.11E-04 | 1.77E-12 | 3.68E-11 | 9.86E-01 | 0.44 | 0.26 |
| CD40 | 1.07E-05 | 5.56E-13 | 3.81E-09 | 4.63E-01 | 0.62 | 0.28 |
| RANTES | 5.25E-02 | 7.99E-12 | 8.40E-10 | 8.72E-01 | 0.23 | 0.24 |
| VEGF | 1.44E-02 | 1.30E-12 | 2.23E-10 | 9.69E-01 | 0.32 | 0.24 |
| VCAM.1 | 7.06E-05 | 9.72E-13 | 4.01E-07 | 4.82E-01 | 0.54 | 0.27 |
| Trefoil.Factor.3..TFF3. | 4.08E-04 | 8.94E-13 | 1.81E-07 | 6.06E-01 | 0.45 | 0.26 |
| Fas.Ligand | 1.07E-01 | 3.62E-11 | 4.65E-10 | 7.43E-01 | 0.17 | 0.23 |
| Gamma.Interferon.induced.Monokine | 4.82E-01 | 8.22E-12 | 2.36E-07 | 9.15E-01 | 0.05 | 0.23 |
| MCP.1 | 5.77E-04 | 1.87E-12 | 2.05E-10 | 5.88E-01 | 0.54 | 0.26 |
| Resistin | 1.13E-01 | 5.76E-12 | 1.78E-09 | 7.38E-01 | 0.12 | 0.23 |
| TIMP.1 | 5.82E-04 | 2.69E-12 | 4.27E-08 | 5.95E-01 | 0.50 | 0.26 |
| SHBG | 5.33E-01 | 6.66E-12 | 1.17E-10 | 8.95E-01 | -0.05 | 0.23 |
| Thyroxine.Binding.Globulin | 3.90E-01 | 5.97E-12 | 3.57E-11 | 9.45E-01 | -0.09 | 0.23 |
| IgA | 5.96E-01 | 1.09E-11 | 5.61E-11 | 9.17E-01 | -0.03 | 0.23 |
| PAI.1 | 4.23E-04 | 1.14E-11 | 9.51E-07 | 3.88E-01 | 0.37 | 0.26 |

| Analyte | *p*-Value | | | | Effect | |
|---|---|---|---|---|---|---|
| | Analyte | APOE Gen. | Age | Sex | Analyte | R Squared |
| Serum.Amyloid.P | 1.39E-01 | 9.10E-12 | 4.07E-11 | 5.03E-01 | -0.12 | 0.23 |
| Myoglobin | 3.57E-01 | 8.76E-12 | 1.94E-10 | 6.74E-01 | 0.04 | 0.23 |
| Apolipoprotein.CIII | 5.26E-01 | 7.71E-12 | 4.42E-11 | 9.90E-01 | -0.06 | 0.23 |
| PAPP.A | 5.42E-01 | 6.95E-12 | 4.96E-11 | 9.98E-01 | -0.08 | 0.23 |
| HCC.4 | 9.00E-01 | 7.81E-12 | 1.31E-10 | 9.56E-01 | -0.01 | 0.23 |
| Prolactin | 6.08E-01 | 8.19E-12 | 4.50E-11 | 8.64E-01 | -0.07 | 0.23 |
| Apolipoprotein.A1 | 1.17E-01 | 1.73E-11 | 1.75E-11 | 8.42E-01 | -0.15 | 0.23 |
| C.Reactive.Protein | 1.54E-01 | 3.39E-10 | 2.83E-11 | 8.97E-01 | -0.05 | 0.23 |
| Complement.3 | 1.71E-02 | 1.37E-11 | 2.76E-08 | 5.24E-01 | 0.23 | 0.24 |
| Fibrinogen | 1.57E-01 | 2.25E-11 | 9.35E-10 | 6.39E-01 | 0.10 | 0.23 |
| Alpha.1.Microglobulin | 9.62E-01 | 7.77E-12 | 5.01E-10 | 9.40E-01 | 0.00 | 0.23 |
| Thrombomodulin | 3.40E-01 | 9.34E-12 | 1.97E-10 | 7.98E-01 | 0.12 | 0.23 |
| Prostatic.Acid.Phosphatase | 3.14E-01 | 1.29E-11 | 7.00E-11 | 9.26E-01 | 0.11 | 0.23 |
| PLGF | 7.03E-01 | 7.22E-12 | 1.12E-10 | 9.87E-01 | -0.04 | 0.23 |
| Apolipoprotein.D | 6.47E-02 | 2.56E-11 | 1.09E-09 | 4.88E-01 | 0.23 | 0.24 |
| Alpha.1.Antitrypsin | 8.28E-01 | 8.05E-12 | 1.22E-10 | 9.67E-01 | -0.03 | 0.23 |
| IP.10..Inducible.Protein.10. | 2.52E-02 | 1.11E-11 | 1.37E-09 | 8.98E-01 | 0.18 | 0.24 |
| Apolipoprotein.H | 9.24E-01 | 7.98E-12 | 7.28E-11 | 9.49E-01 | -0.01 | 0.23 |
| MIP.1beta | 9.50E-03 | 1.61E-12 | 2.02E-10 | 9.08E-01 | 0.28 | 0.24 |
| SGOT | 7.64E-01 | 8.81E-12 | 9.17E-11 | 9.22E-01 | 0.03 | 0.23 |
| *ADNI* | | | | | | |
| Fatty.Acid.Binding.Protein | 7.40E-18 | 6.08E-18 | 2.38E-01 | 5.32E-01 | 0.50 | 0.44 |
| TNF.RII | 6.11E-07 | 2.38E-21 | 1.15E-01 | 8.70E-01 | 0.55 | 0.33 |
| Tissue.Factor | 4.20E-09 | 2.30E-21 | 1.94E-01 | 9.06E-01 | 0.46 | 0.36 |
| Hepatocyte.Growth.Factor..HGF. | 5.13E-14 | 7.71E-22 | 3.79E-01 | 7.68E-01 | 0.66 | 0.40 |
| MMP.3 | 5.04E-05 | 1.25E-21 | 3.86E-01 | 9.75E-01 | 0.33 | 0.31 |
| CgA | 1.27E-07 | 2.79E-22 | 5.97E-01 | 1.54E-01 | 0.95 | 0.34 |
| Stem.Cell.Factor | 2.97E-05 | 3.65E-22 | 7.44E-01 | 8.07E-01 | 0.43 | 0.31 |
| Sortilin | 9.10E-04 | 2.97E-21 | 7.64E-01 | 4.42E-01 | 0.50 | 0.30 |
| LOX.1 | 1.34E-05 | 2.40E-21 | 2.11E-01 | 5.90E-01 | 0.47 | 0.32 |
| Beta.2.Microglobulin | 5.07E-05 | 1.46E-22 | 2.54E-01 | 9.82E-01 | 0.53 | 0.31 |
| TRAIL.R3 | 4.73E-04 | 7.19E-22 | 2.21E-01 | 6.53E-01 | 0.38 | 0.30 |
| Angiopoietin.2..ANG.2. | 1.90E-04 | 4.13E-22 | 3.42E-01 | 9.48E-01 | 0.36 | 0.31 |
| NT.proBNP | 1.06E-03 | 1.39E-21 | 2.59E-01 | 3.09E-01 | 0.36 | 0.30 |
| Alpha.2.Macroglobulin | 2.96E-03 | 7.26E-22 | 4.29E-01 | 8.84E-01 | 0.39 | 0.30 |
| HB.EGF | 8.84E-04 | 1.20E-21 | 5.29E-01 | 5.87E-01 | 0.49 | 0.30 |
| Osteopontin | 8.58E-02 | 1.01E-20 | 9.14E-01 | 3.70E-01 | 0.18 | 0.28 |
| Cystatin.C | 1.40E-06 | 1.29E-22 | 3.91E-01 | 8.67E-01 | -0.93 | 0.33 |
| Apolipoprotein.E | 8.26E-04 | 1.48E-23 | 6.84E-01 | 8.66E-01 | 0.40 | 0.30 |
| ACE | 7.86E-04 | 2.62E-22 | 4.49E-01 | 4.18E-01 | 0.34 | 0.30 |
| S100b | 1.92E-03 | 2.24E-19 | 6.09E-01 | 5.71E-01 | 0.33 | 0.30 |
| Clusterin..Apo.J. | 4.30E-03 | 4.09E-21 | 4.75E-01 | 7.32E-01 | 0.29 | 0.29 |
| TGF.alpha | 2.33E-03 | 4.65E-22 | 9.47E-01 | 1.22E-01 | 0.25 | 0.31 |
| von.Willebrand.Factor | 4.02E-03 | 1.55E-21 | 2.03E-01 | 5.85E-01 | 0.31 | 0.29 |
| MIF | 8.15E-03 | 1.32E-20 | 7.37E-01 | 4.63E-01 | 0.14 | 0.29 |
| Ferritin | 5.10E-03 | 1.66E-19 | 6.30E-01 | 7.07E-01 | 0.30 | 0.29 |

| Analyte | p-Value | | | | Effect | |
|---|---|---|---|---|---|---|
| | Analyte | APOE Gen. | Age | Sex | Analyte | R Squared |
| AXL | 1.12E-02 | 1.42E-21 | 7.16E-01 | 5.01E-01 | 0.27 | 0.29 |
| IGF.BP.2 | 1.48E-02 | 2.33E-22 | 4.42E-01 | 7.65E-01 | 0.55 | 0.30 |
| Adiponectin | 6.66E-03 | 4.01E-22 | 6.24E-01 | 6.07E-01 | 0.24 | 0.29 |
| Cortisol | 2.62E-02 | 1.12E-21 | 5.30E-01 | 8.11E-01 | 0.24 | 0.30 |
| IL.6.Receptor | 2.53E-02 | 2.81E-21 | 6.41E-01 | 5.05E-01 | 0.25 | 0.29 |
| CD40 | 2.60E-02 | 8.52E-22 | 3.92E-01 | 4.94E-01 | 0.31 | 0.29 |
| RANTES | 3.67E-03 | 7.56E-21 | 7.17E-01 | 4.76E-01 | 0.15 | 0.29 |
| VEGF | 3.55E-02 | 4.01E-22 | 5.79E-01 | 7.12E-01 | 0.28 | 0.28 |
| VCAM.1 | 9.86E-03 | 6.86E-22 | 3.83E-01 | 9.41E-01 | 0.32 | 0.29 |
| Trefoil.Factor.3..TFF3. | 1.56E-01 | 9.77E-22 | 5.39E-01 | 7.38E-01 | 0.14 | 0.28 |
| Fas.Ligand | 3.07E-01 | 7.44E-20 | 7.73E-01 | 4.82E-01 | 0.11 | 0.28 |
| Gamma.Interferon.induced.Monokine | 8.52E-01 | 7.42E-21 | 9.90E-01 | 3.82E-01 | -0.01 | 0.27 |
| MCP.1 | 3.57E-01 | 2.19E-21 | 8.09E-01 | 6.72E-01 | 0.12 | 0.28 |
| Resistin | 1.45E-01 | 7.93E-21 | 7.62E-01 | 5.85E-01 | 0.09 | 0.28 |
| TIMP.1 | 6.23E-01 | 2.11E-21 | 8.21E-01 | 5.38E-01 | 0.07 | 0.27 |
| SHBG | 2.21E-01 | 1.24E-21 | 7.41E-01 | 4.46E-01 | 0.08 | 0.28 |
| Thyroxine.Binding.Globulin | 4.97E-01 | 1.81E-21 | 8.96E-01 | 5.63E-01 | 0.05 | 0.27 |
| IgA | 6.16E-01 | 2.02E-21 | 8.97E-01 | 5.62E-01 | 0.03 | 0.27 |
| PAI.1 | 3.17E-01 | 1.86E-21 | 7.36E-01 | 6.22E-01 | 0.10 | 0.28 |
| Serum.Amyloid.P | 8.31E-01 | 2.24E-21 | 9.03E-01 | 5.46E-01 | 0.01 | 0.27 |
| Myoglobin | 4.04E-01 | 1.61E-21 | 8.63E-01 | 6.05E-01 | 0.03 | 0.28 |
| Apolipoprotein.CIII | 4.97E-01 | 1.81E-21 | 8.39E-01 | 4.82E-01 | 0.05 | 0.27 |
| PAPP.A | 4.61E-01 | 6.25E-21 | 7.71E-01 | 6.00E-01 | 0.09 | 0.28 |
| HCC.4 | 8.18E-01 | 2.28E-21 | 9.00E-01 | 4.91E-01 | 0.02 | 0.27 |
| Prolactin | 4.88E-01 | 1.80E-21 | 9.46E-01 | 5.10E-01 | -0.09 | 0.27 |
| Apolipoprotein.A1 | 4.19E-01 | 1.92E-21 | 8.46E-01 | 5.35E-01 | 0.06 | 0.27 |
| C.Reactive.Protein | 8.26E-02 | 1.13E-19 | 9.80E-01 | 5.32E-01 | -0.05 | 0.28 |
| Complement.3 | 8.24E-01 | 2.28E-21 | 8.91E-01 | 4.87E-01 | 0.02 | 0.27 |
| Fibrinogen | 3.65E-01 | 2.50E-21 | 9.83E-01 | 3.43E-01 | -0.04 | 0.28 |
| Alpha.1.Microglobulin | 9.49E-01 | 3.28E-21 | 9.11E-01 | 4.87E-01 | 0.01 | 0.27 |
| Thrombomodulin | 6.47E-01 | 3.63E-21 | 9.92E-01 | 3.93E-01 | -0.05 | 0.27 |
| Prostatic.Acid.Phosphatase | 3.45E-01 | 1.47E-21 | 8.78E-01 | 4.60E-01 | 0.08 | 0.28 |
| PLGF | 2.60E-01 | 2.65E-21 | 7.76E-01 | 2.60E-01 | -0.10 | 0.28 |
| Apolipoprotein.D | 7.88E-01 | 5.58E-21 | 9.70E-01 | 4.09E-01 | -0.03 | 0.27 |
| Alpha.1.Antitrypsin | 6.80E-01 | 2.33E-21 | 9.97E-01 | 3.96E-01 | -0.05 | 0.27 |
| IP.10..Inducible.Protein.10. | 9.89E-01 | 5.68E-21 | 9.22E-01 | 4.38E-01 | 0.00 | 0.27 |
| Apolipoprotein.H | 5.62E-01 | 3.80E-21 | 9.94E-01 | 3.49E-01 | -0.05 | 0.27 |
| MIP.1beta | 5.22E-01 | 1.47E-19 | 9.44E-01 | 5.72E-01 | 0.06 | 0.26 |
| SGOT | 8.21E-01 | 2.72E-21 | 9.28E-01 | 4.37E-01 | 0.03 | 0.27 |

ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRC, Alzheimer's Disease Research Center; CSF, cerebrospinal fluid; Gen., genotype; QC, quality control.

**Table S4.** Association of CSF analytes with the CSF ptau-A$\beta_{42}$ ratio for cognitively impaired subjects*.

| | Cognitive Declined (*n* = 303) | | |
|---|---|---|---|
| | *p*-Value | Effect | CI |
| Fatty Acid Binding Protein (Heart) - H-FABP | 2.19E-16 | 0.47 | (0.36-0.57) |
| Tumor Necrosis Factor Receptor 2 - TNFR2 | 3.04E-10 | 0.67 | (0.47-0.87) |
| Tissue Factor - F3 | 3.68E-12 | 0.52 | (0.38-0.66) |
| Hepatocyte Growth Factor - HGF | 1.49E-12 | 0.63 | (0.46-0.80) |
| Chromogranin A - CgA | 3.81E-13 | 1.23 | (0.91-1.55) |
| Stem Cell Factor - KITLG | 3.51E-08 | 0.55 | (0.36-0.75) |
| Sortilin - SORT1 | 4.34E-05 | 0.61 | (0.32-0.90) |
| Lectin Like Oxidized LDL Receptor 1 - LOX1 | 5.71E-05 | 0.40 | (0.20-0.59) |
| Beta$_2$Microglobulin (B2M) | 4.35E-06 | 0.57 | (0.33-0.81) |
| TNF-Related Apoptosis-Inducing Ligand R3 - TRAIL-R3 | 1.38E-02 | 0.28 | (0.06-0.50) |
| Angiopoietin 2 - ANGPT2 | 1.05E-04 | 0.38 | (0.19-0.57) |
| Apolipoprotein E - APOE | 3.96E-07 | 0.52 | (0.32-0.72) |
| Angiotensin-Converting Enzyme - ACE | 8.42E-05 | 0.38 | (0.19-0.57) |

*Includes all the subjects with CDR > 0 regardless of their CSF A$\beta_{42}$ levels.
CDR, Clinical Dementia Rating scale; CI, confidence interval; CSF, cerebrospinal fluid.

**Table S5.** Plasma analytes levels. Association results with CSF ptau-A$\beta_{42}$ ratio, and correlation to CSF levels.

| | Association with CSF ptau/A$\beta_{42}$ | | | | Pearson's Correlation | |
|---|---|---|---|---|---|---|
| | Knight-ADRC | | ADNI | | Knight-ADRC | ADNI |
| | *p*-Value | Effect | *p*-Value | Effect | R2 | R2 |
| Fatty Acid Binding Protein (Heart) - H-FABP | 9.61E-01 | 0.00 | 9.61E-01 | -0.01 | 0.32 | 0.23 |
| Tumor Necrosis Factor Receptor 2 - TNFR2 | 4.05E-01 | -0.11 | 6.77E-01 | -0.09 | 0.10 | 0.37 |
| Tissue Factor - F3 | - | - | - | - | - | - |
| Hepatocyte Growth Factor - HGF | 4.01E-01 | 0.14 | 9.33E-01 | -0.03 | 0.15 | 0.01 |
| Chromogranin A - CgA | 7.48E-01 | -0.02 | 1.36E-01 | -0.15 | 0.15 | -0.10 |
| Stem Cell Factor - KITLG | 7.29E-01 | 0.04 | 7.34E-01 | 0.07 | -0.01 | 0.03 |
| Sortilin - SORT1 | 6.22E-01 | 0.08 | 2.43E-01 | -0.40 | 0.05 | 0.04 |
| Lectin Like Oxidized LDL Receptor 1 - LOX1 | - | - | 2.43E-01 | -0.40 | - | 0.04 |
| Beta2Microglobulin (B2M) | 5.15E-01 | 0.12 | 7.74E-01 | 0.09 | 0.25 | 0.15 |
| TNF (TRAIL-R3) | 4.05E-01 | -0.11 | 3.55E-01 | -0.22 | 0.22 | 0.12 |
| Angiopoietin 2 - ANGPT2 | 2.79E-01 | 0.14 | 6.39E-01 | 0.13 | 0.22 | 0.12 |
| Cystatin C - CST3 | 8.30E-01 | 0.04 | 9.53E-01 | -0.02 | 0.19 | -0.12 |
| Apolipoprotein E - APOE | 5.55E-01 | -0.08 | 2.30E-01 | 0.29 | 0.10 | 0.12 |
| Angiotensin-Converting Enzyme - ACE | 6.96E-02 | -0.18 | 3.48E-01 | -0.24 | 0.19 | 0.27 |

ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRC, Alzheimer's Disease Research Center; CSF, cerebrospinal fluid.

**Table S6.** CSF analytes significantly associated with CSF ptau-A$\beta_{42}$ ratio, corrected by CSF H-FABP, and CSF APOE.

| | H-FABP Corrected | | | | APOE Corrected | | | |
| | WU | | ADNI | | WU | | ADNI | |
| Analyte | *p*-Value | Effect | *p*-Value | Effect | *p*-Value | Effect | *p*-Value | Effect |
|---|---|---|---|---|---|---|---|---|
| H-FABP | - | - | - | - | 1.34E-14 | 0.59 | 1.58E-15 | 0.51 |
| TNFR2 | 1.09E-01 | 0.28 | 3.28E-01 | 0.11 | 7.58E-10 | 1.02 | 2.19E-04 | 0.54 |
| F3 | 2.86E-01 | 0.12 | 3.90E-01 | 0.08 | 5.23E-10 | 0.72 | 1.32E-06 | 0.49 |
| HGF | 7.92E-02 | 0.28 | 4.04E-06 | 0.41 | 2.37E-07 | 0.90 | 1.48E-11 | 0.64 |
| CgA | 8.57E-02 | 0.34 | 1.76E-01 | 0.25 | 7.75E-07 | 1.12 | 2.86E-05 | 0.85 |
| KITLG | 8.96E-01 | 0.02 | 4.61E-01 | 0.08 | 3.36E-05 | 0.56 | 1.93E-03 | 0.34 |
| SORT1 | 1.50E-01 | 0.25 | 5.20E-01 | 0.09 | 1.21E-04 | 0.78 | 6.73E-02 | 0.32 |
| LOX1 | 9.03E-01 | -0.01 | 8.06E-01 | -0.03 | 4.51E-04 | 0.42 | 1.78E-03 | 0.39 |
| (B2M) | 6.13E-01 | -0.08 | 9.68E-01 | -0.01 | 1.52E-03 | 0.60 | 1.29E-02 | 0.42 |
| TNF (TRAIL-R3) | 1.04E-01 | 0.27 | 6.02E-01 | 0.05 | 1.93E-04 | 0.66 | 1.30E-02 | 0.28 |
| ANGPT2 | 9.48E-01 | 0.01 | 7.94E-01 | 0.03 | 6.67E-03 | 0.45 | 2.32E-02 | 0.26 |
| APOE | 3.03E-01 | -0.13 | 6.84E-01 | -0.05 | - | - | - | - |
| ACE | 5.39E-02 | -0.22 | 5.61E-01 | -0.06 | 5.48E-02 | 0.24 | 5.60E-02 | 0.23 |

ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; CSF, cerebrospinal fluid; H-FABP, fatty acid binding protein; WU, Washington University.

**Table S7.** Association of CSF H-FABP with CDR sum of boxes and Mini-Mental State Examination.

| | At Lumbar Puncture | | | Latest Available | | |
| Study | *p*-Value | Effect | 95% CI | *p*-Value | Effect | 95% CI |
|---|---|---|---|---|---|---|
| **CDR - sum of boxes** | | | | | | |
| Knight-ADRC | 6.00E-02 | 0.37 | (-0.02-0.76) | 2.46E-02 | 0.85 | (0.11-1.60) |
| ADNI | 3.51E-04 | 0.58 | (0.27-0.90) | 2.61E-03 | 1.24 | (0.43-2.04) |
| **MMSE** | | | | | | |
| Knight-ADRC | 1.02E-01 | -0.48 | (-1.06-0.10) | 1.39E-02 | -1.29 | (-2.32--0.26) |
| ADNI | 4.79E-04 | -0.83 | (-1.30--0.37) | 1.65E-03 | -1.88 | (-3.05--0.72) |

ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRC, Alzheimer's Disease Research Center; CDR, Clinical Dementia Rating scale; CI, confidence interval; CSF, cerebrospinal fluid; H-FABP, fatty acid binding protein; MMSE, Mini-Mental State Examination.

**Table S8.** Importance of the CSF analytes estimated by the random forest method

| Knight-ADRC | | ADNI | |
|---|---|---|---|
| **Analyte** | **Importance** | **Analyte** | **Importance** |
| Fatty Acid Binding Protein | 19.19247246 | Fatty Acid Binding Protein | 8.502826081 |
| TNF RII | 6.666840852 | Hepatocyte Growth Factor | 7.307953027 |
| Tissue Factor | 5.963167857 | Osteopontin | 3.293181286 |
| MIF | 5.815442597 | Tissue Factor | 2.977846024 |
| Hepatocyte Growth Factor | 4.827981009 | Tissue Inhibitor of Metalloproteinases 1 | 2.559904355 |
| LOX 1 | 4.655182008 | Chromogranin A | 2.497783151 |
| VEGF | 4.091851486 | Fibrinogen | 2.376966156 |
| CgA | 3.713313639 | Macrophage Migration Inhibitory Factor | 2.066185355 |
| Apolipoprotein A1 | 3.261947852 | Interferon gamma Induced Protein 10 | 1.969275387 |
| TRAIL R3 | 3.054363712 | Matrix Metalloproteinase 3 | 1.891908229 |
| Ferritin | 2.937114472 | Apolipoprotein D | 1.683746502 |
| Angiopoietin 2 | 2.929077784 | Alpha 1 Antitrypsin  AAT | 1.668720973 |
| SHBG | 2.681659547 | Monocyte Chemotactic Protein 1 | 1.546074605 |
| Thyroxine Binding Globulin | 2.660406657 | TNF R2 | 1.480163578 |
| Sortilin | 2.621608522 | Prolactin | 1.474023529 |
| MCP 1 | 2.590687472 | Immunoglobulin A  IgA | 1.458514488 |
| Alpha 1 Antitrypsin | 2.354561739 | CD 40 antigen  CD40 | 1.423424543 |
| C Reactive Protein | 2.332054331 | Cystatin C | 1.418272538 |
| Myoglobin | 2.239611823 | Prostatic Acid Phosphatase  PAP | 1.407672138 |
| Alpha 1 Microglobulin | 2.14188654 | Tumor Necrosis Factor Receptor 2 | 1.310757202 |
| Thrombomodulin | 2.038534421 | Plasminogen Activator Inhibitor 1 | 1.28601234 |
| PAPP A | 1.987617215 | Placenta Growth Factor | 1.266546889 |
| MIP 1beta | 1.981963203 | RANTES | 1.239050257 |
| VCAM 1 | 1.978115933 | N terminal prohormone of brain natriuret | 1.238322203 |
| Apolipoprotein E | 1.970156681 | Vascular Endothelial Growth Factor | 1.206003982 |
| PLGF | 1.957949048 | von Willebrand Factor  vWF | 1.195490215 |
| AXL | 1.869455575 | Ferritin | 1.18010753 |
| Serum Amyloid P | 1.858635482 | Alpha 1 Microglobulin | 1.174038728 |
| Fibrinogen | 1.853006174 | Myoglobin | 1.143834953 |
| von Willebrand Factor | 1.767324603 | Alpha 2 Macroglobulin | 1.140555498 |
| Apolipoprotein CIII | 1.765737779 | Lectin Like Oxidized LDL Receptor 1 | 1.124217977 |
| CD40 | 1.715882261 | Apolipoprotein E | 1.053298759 |
| HCC 4 | 1.696730191 | Apolipoprotein H | 1.034575496 |
| MMP 3 | 1.683006665 | Sortilin | 1.029761444 |
| SGOT | 1.673246908 | Adiponectin | 1.019162402 |
| Trefoil Factor 3  TFF3 | 1.657552754 | Sex Hormone Binding Globulin | 1.007111882 |
| Beta 2 Microglobulin | 1.635657724 | AXL Receptor Tyrosine Kinase | 0.998195736 |
| HB EGF | 1.616892551 | Chemokine CC 4 | 0.980921224 |
| Apolipoprotein H | 1.595525938 | Serum Glutamic Oxaloacetic T | 0.96203632 |
| IL 6 Receptor | 1.556260707 | Interleukin 6 receptor | 0.941942317 |
| Adiponectin | 1.494300366 | S100 calcium binding protein B | 0.940847039 |

| Knight-ADRC | | ADNI | |
|---|---|---|---|
| **Analyte** | **Importance** | **Analyte** | **Importance** |
| Alpha 2 Macroglobulin | 1.487908504 | Apolipoprotein C III | 0.917586691 |
| S100b | 1.477834412 | Complement C3 | 0.909654636 |
| Gamma Interferon | 1.438045547 | Angiopoietin 2 | 0.908510854 |
| Apolipoprotein D | 1.426908178 | Heparin Binding EGF | 0.872734408 |
| TIMP 1 | 1.422091242 | ACE | 0.822171482 |
| IGF BP 2 | 1.417433267 | Thyroxine Binding Globulin | 0.816560012 |
| Stem Cell Factor | 1.400511511 | Serum Amyloid P Component | 0.795180325 |
| NT proBNP | 1.392725055 | Beta 2 Microglobulin | 0.786526162 |
| PAI 1 | 1.384777834 | Trefoil Factor 3 | 0.757941479 |
| Prolactin | 1.344548498 | Apolipoprotein A I | 0.728521975 |
| Complement 3 | 1.304572125 | Clusterin | 0.679869294 |
| TGF alpha | 1.206086518 | Vascular Cell Adhesion Molecule 1 | 0.579263316 |

ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRC, Alzheimer's Disease Research Center; CSF, cerebrospinal fluid.

## Supplemental References

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