Supplementary Online Content

Sutphen CL, Jasielec MS, Shah AR, et al. Longitudinal cerebrospinal fluid biomarker changes in preclinical Alzheimer disease during middle age. *JAMA Neurol*. Published online July 6, 2015. doi:10.1001/jamaneurol.2015.1285.

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable. Assay performance and specifications as provided by the vendors.

Vendor	Fujirebio (formerly Innogenetics)				EUROIMMUN			QUIDEL	SINGULEX
Analyte	**Aß1-40**	**Aß1-42**	**Total tau**	**Ptau181**	**Aß1-40**	**Aß1-42**	**Total tau**	**YKL-40**	**VILIP-1**
Technology	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	ELISA Colorimetric	Single Molecule Counting (SMC™)
Fluid Sample	CSF	CSF	CSF	CSF	CSF	CSF	CSF	CSF	CSF
Capture Antibody	2G3	21F12	AT120	HT7	2G3	21F12	ADx 201	Not Available	3A8.1
Capture Antibody Epitope	35-40	37-42	218-224	159-163	Aßx-40	Aßx-42	Proline-rich region	Not Available	Not Available
Detection Antibody	3D6	3D6	HT7/BT2	AT270	3D6	3D6	ADx 215	Not Available	Sheep 22
Detection Antibody Epitope	1-5	1-5	159-163/193-198	176-182	Aß1-x	Aß1-x	N-terminus	Not Available	Multiple
Calibrator Concentration Range, pg/mL ***(lot specific, for this study)***	7.8 - 1000 (n=8)	62.5 - 4000 (n=6)	50 - 2500 (n=6)	15.6 - 1000 (n=6)	54 - 711 (n=6)	83 - 1236 (n=6)	30 - 1050 (n=6)	20 - 300 (n=6)	3.9 - 3000 (n=10)
Limit of Detection (LOD), pg/mL, (range), number of runs	3.3 (0.7 - 4.2) 4 runs	65 (52-87) 7 runs	34 (25-47) 8 runs	13.4 (11.4 - 14.9) 4 runs	70 (58 - 85) 6 runs	7.5 (6.3 - 9.0) 6 runs	44.2 (41.1 - 46.8) 4 runs	5.4 Not available Not available	1.4 (0.077-6.9) 16 runs
Sample Specifications									
Sample Pre-dilution	1:100	No	No	No	1:21	No	No	1:03	No
Volume Sample Per Well (µL)	75	25	25	75	15	15	25	20	15
Total Well Volume	100	100	100	100	115	115	125	120	200
Number of Replicate Wells	2	2	2	2	2	2	2	2	2
Sample Incubation									
Time, hrs	14-18	1	14-18	14-18	3	3	3	1	2
Temperature	2-8°C	23-27°C	23-27°C	2-8°C	23-27°C	23-27°C	23-27°C	23-27°C	23-27°C
Reported Intra-Assay Variability[1], % CV, (range), number of runs	2.8 (0.3 - 7.8) 5 runs	4.6 (0.8 - 11.0) 3 runs	3.2 (0.0 - 13.2) 2 runs	1.7 (0.0 - 8.9) 4 runs	2.7 (2.4 - 3.1) 3 runs	3.9 (2.3 - 6.2) 3 runs	5.0 (3.9 - 6.5) 4 runs	6 (5.6 - 6.6) Not available	4.4 (3.1 - 7.0) 10 runs
Reported Inter-Assay Variability[1], %CV, (range), number of runs	4.4 (3.2 - 5.8) 4 runs	7.8 (1.4 - 18.0) 3 runs	11.5 (3.8 - 42.8) 2 runs	11.4 (6.6 - 19.2) 4 runs	8.3 (7.3 - 9.3) 5 runs	5.6 (4.4 - 7.6) 7 runs	7.4 (6.1 - 9.3) 5 runs	6.7 (6.0 - 7.0) Not available	6.2 (5.0 - 7.0) 1 run
Study Intra-Assay Variability[2], % CV, (range), number of runs	2.7 (0.4 - 12.7) 12 runs	4.4 (0.2 - 13.7) 12 runs	2.0 (0.0 - 11.4) 12 runs	1.0 (0.1 - 4.6) 12 runs	2.3 (0.1 - 6.0) 12 runs	3.0 (0.1 - 9.7) 12 runs	5.42 (0.8 - 22.9) 12 runs	1.9 (0.1 -6.4) 12 runs	4.5 (0.0 - 20.8) 16 runs
Study Inter-Assay Variability[2], %CV, (range), number of runs	3.8 (0.0 - 24.6) 12 runs	4.3 (0.0 - 20.6) 12 runs	2.1 (0.0 - 14.8) 12 runs	2.2 (0.0 - 17.5) 12 runs	14 (0.1 - 13.7) 12 runs	4.1 (0.0 - 16.7) 12 runs	4.1 (0.0 - 24.2) 12 runs	3.7 (0.0 - 21.6) 12 runs	4.3 (0.0 - 24.8) 12 runs

Samples were analyzed for Aβ1-40, Aβ1-42, total tau, and phosphorylated-tau181 (ptau181) using the "Improved" INNOTEST™ ELISA (Fujirebio Europe [formerly Innogenetics], Gent, Belgium). Compared to the previous version of the INNOTEST™ assays, these improved assays each contain a set of ready-to-use calibrator series, run validation control samples (= calibrator in buffer), and harmonized buffer reagents. For comparison purposes, concentrations of Aβ1-40, Aβ1-42 and total tau were also measured using EUROIMMUN ELISAs (EUROIMMUN, Luebeck, Germany). The EUROIMMUN assays are considered second generation assays since they do not possess matrix interference (i.e., they exhibit good dilutional linearity). For Aß species, both assays utilize the same monoclonal antibodies, but they are obtained from a different process of culture, production, and purification. In addition, the amount of CSF included in the wells for testing is lower in the EUROIMMUN assays, as such reducing the probable issues with matrix interference[1, 2]. So in principle, the EUROIMMUN assay measures another fraction of the free, non-protein bound analyte, in a sample. YKL-40 (also known as chitinase-3 like 1), an astrocyte-derived marker of gliosis/neuroinflammation, was measured with the MicroVue ELISA (Quidel, San Diego, CA), as described previously[3]. Visinin-like protein 1 (VILIP-1), a marker of neuronal injury, was measured using a two-site immunoassay implemented via a microparticle-based Erenna immunoassay system (Singulex, Alameda, CA) as described previously[4]. All ELISA kits passed in-house quality control measures including: 1) standard curve values having a CV <25% and no more than two standards either oversaturating or undersaturating using a Synergy 2 Multi-Mode Reader (Bio-Tek Instruments, Inc.), 2) no more than one provided kit control and one internal pooled CSF control failing due to reading outside the provided range or having a CV >25% with the exception of one assay in which both internal CSF controls failed due to high CV – in this case, both kit controls passed, and 3) samples failed if there was a CV >25%. The Singulex kits passed in-house quality control measures including: 1) standard curves were assessed according to software provided by Singulex, 2) no more than 2 of 3 internal pooled CSF

controls falling outside the range determined by all previous runs using the same lot number of kits and 3) samples failed if there was a CV >25%. [1] performance characteristics as provided by the vendor. [2] actual performance characteristics in the current study.

eReferences

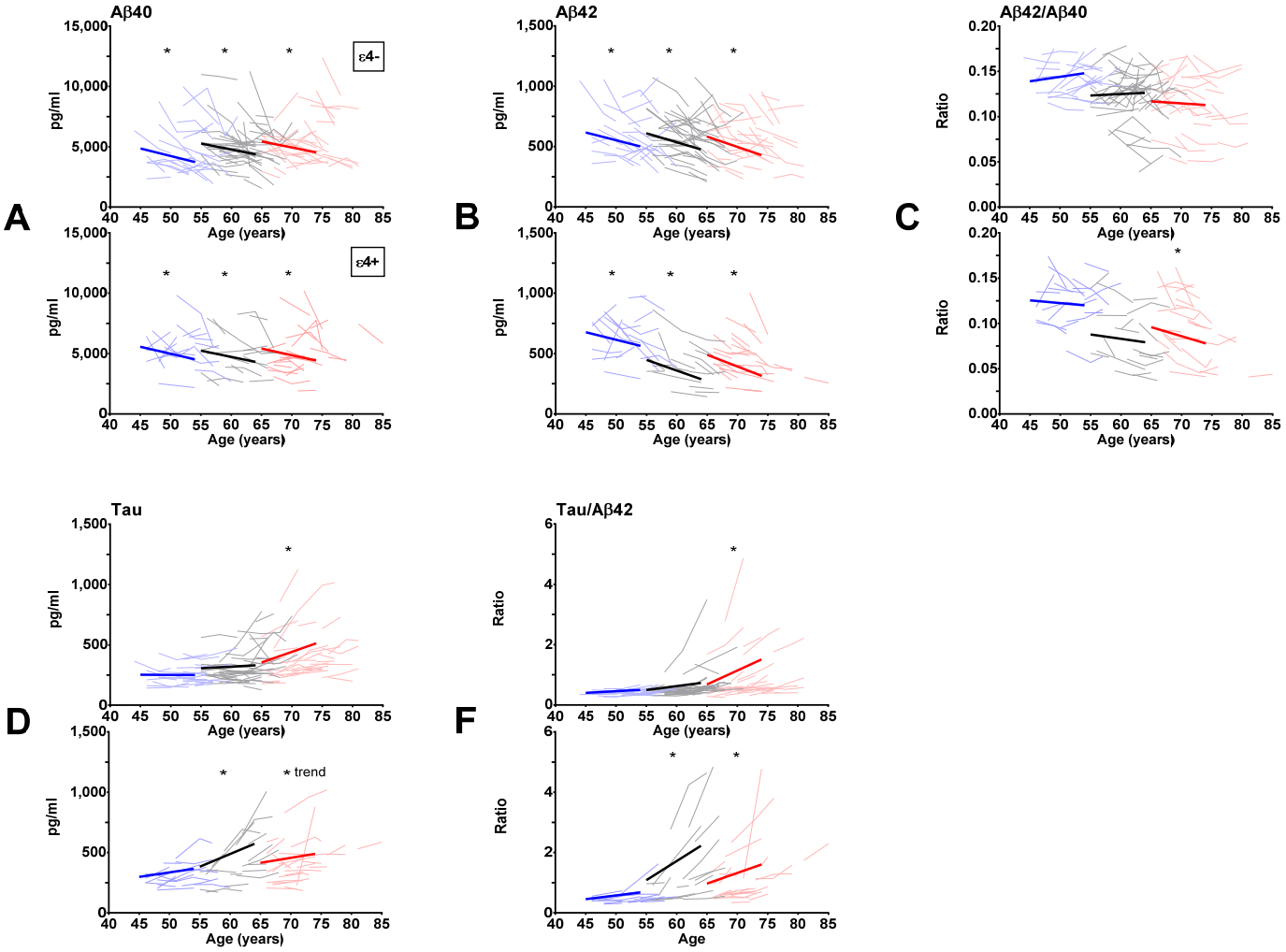
1. Bjerke M, Portelius E, Minthon L, et al. Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis*. 2010;2010.

2. Cullen VC, Fredenburg RA, Evans C, Conliffe PR, Solomon ME. Development and advanced validation of an optimized method for the quantitation of Abeta42 in human cerebrospinal fluid. *AAPS J*. Sep 2012;14(3):510-8.

3. Craig-Schapiro R, Perrin RJ, Roe CM, et al. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry*. Nov 15 2010;68(10):903-12.

4. Tarawneh R, D'Angelo G, Macy E, et al. Visinin-like protein-1: diagnostic and prognostic biomarker in Alzheimer disease. *Ann Neurol*. Aug 2011;70(2):274-85.

eFigure 1. Group longitudinal change over spaghetti plots of EUROIMMUN CSF biomarkers during middle-age. Estimated slopes and within-person patterns for A) Aβ40, B) Aβ42, C) the Aβ42/Aβ40 ratio, D) tau, and E) tau/Aβ42 are shown in the three age bins for *APOE* ε4-negative (top graph of each panel, n=108participants) and ε4-positive (bottom graph of each panel, n=61 participants) groups. Annual slopes have been extrapolated to 9 years, and each slope begins at the mean baseline biomarker value of each age bin. Blue, EARLY middle-age (45-54 years at baseline); Black, MID middle-age (55-64 years at baseline); Red, LATE middle-age (65-74 years at baseline). * slopes significantly different from 0 ($p<0.05$). ‡, slopes significantly different between *APOE* ε4 groups within a given age group ($p<0.05$). Trend, p=0.051-0.06.



eFigure 2. Longitudinal biomarker trajectories in individuals with different *APOE* genotypes. Within-person trajectories of CSF A) Aβ40, B) Aβ42, C) tau, D) the tau/Aβ42 ratio, E) VILIP-1, and F) YKL-40 are plotted as a function of age. Aβ40, Aβ42 and tau were measured with the INNOTEST assay. Colors identify *APOE* genotype: no ε4 alleles (gray, ε4-/-; ε2/ε2, ε2/ε3, ε3/ε3), one ε4 allele (purple, ε4+/-; ε2/ε4, ε3/ε4) and two ε4 alleles (orange, ε4+/+; ε4/ε4).