Supplementary Material

Detection and Quantification of Aβ–3–40 (APP669-711) in Cerebrospinal Fluid

Hans-Wolfgang Klafki*, Oliver Wirths, Brit Mollenhauer, Thomas Liepold, Petra Rieper,
Hermann Esselmann, Jonathan Vogelgsang, Jens Wiltfang, Olef Jahn*

©Department of Psychiatry and Psychotherapy, University Medical Center Goettingen, Georg-August-
University, Goettingen, Germany

©Department of Neurology, University Medical Center Goettingen, Georg-August-University
Goettingen, Germany

©Paracelsus-Elena-Klinik, Kassel, Germany

©Max Planck Institute of Experimental Medicine, Proteomics Group, Goettingen, Germany

©German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany

©Neurosciences and Signaling Group, Institute of Biomedicine (iBiMED), Department of Medical
Sciences, University of Aveiro, Aveiro, Portugal

©These authors contributed equally

*Correspondence to Hans-W. Klafki or Olaf Jahn, Dept. of Psychiatry and Psychotherapy, University
Medical Center Goettingen, Georg-August-University, Von-Siebold-Str. 5, D37075 Goettingen,
Germany; e-mail: hans.klafki@med.uni-goettingen.de, tel.: +49 5513965797 or olaf.jahn@med.uni-
goettingen.de, tel. +49 5513899313

©Current address: McLean Hospital, Department of Psychiatry, Harvard Medical School, Translational
Neuroscience Laboratory, Belmont, MA, 02478, USA

Content:

Supplementary Figures 1-4
Supplementary Table 1
Supplementary Figure 1. Schematic workflow for detection and quantification of Aβ−3−40 in cerebrospinal fluid (CSF). Left column: Amyloid-β (Aβ)−3−40 was identified by an immunoprecipitation-mass spectrometry (IP-MS) method comprising two-step immuno-enrichment followed by analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The method was first established with supernatants from transfected SH-SY5Y cells stably overexpressing wildtype amyloid precursor protein (APP)695, in which the presence of Aβ−3−40 is documented, and then applied to pooled lumbar or normal pressure hydrocephalus (NPH) cerebrospinal fluid (CSF) samples. Right column: Aβ−3−40 was quantified directly from diluted CSF samples by an established electrochemiluminescence sandwich immunoassay. The study cohort included 23 amyloid PET-negative (Aβ−) and 17 amyloid PET-positive (Aβ+) participants. Using existing data on the CSF levels of Aβ40 and Aβ42 from the same cohort, different CSF Aβ ratios and a composite score were evaluated for their potential as biomarker candidates on the basis of receiver operating characteristic (ROC) curves.
Supplementary Figure 2. MALDI-TOF-MS mass spectra of immunoprecipitated Aβ peptides from cell culture supernatant. Aliquots of conditioned cell culture medium of SH-SY5Y cells overexpressing human amyloid precursor protein (APP)695 were subjected to two consecutive rounds of magnetic bead immunoprecipitation (IP) and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In the first IP-round, the samples were incubated with mAb 6E10 covalently coupled to Dynabeads M-280 Sheep anti-Mouse IgG overnight at approx. 4°C (mass spectra I, III, V) or for 1 hour at room temperature (II, IV, VI). In the second IP-round (1 hour at room temperature), Dynabeads M-270 Epoxy functionalized with mAb 6E10 (I, II), mAb 101-1-1 (III, IV) or mAb 14-2-4 (V, VI) were employed. Note the different scaling of the intensity axis in I and II vs. III-VI. Reflector mode mass spectra are shown with annotations of the identified respective amyloid-β (Aβ) peptides on top. The signals for Aβ-3-40 are highlighted in red.
Supplementary Figure 3. MALDI-TOF-MS mass spectra of immunoprecipitated Aβ peptides from NPH-CSF. Aliquots of normal pressure hydrocephalus cerebrospinal fluid (NPH-CSF) were subjected to two consecutive rounds of magnetic bead immunoprecipitation (IP) and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In the first IP-round, the samples were incubated with mAb 6E10 covalently coupled to Dynabeads M-280 Sheep anti-Mouse IgG overnight at approx. 4°C. In the second IP-round (1 hour at room temperature), Dynabeads M-270 Epoxy functionalized with mAb 14-2-4 (A, mass spectra I and II) or mAb 101-1-1 (A, III) were employed. The mass spectra II and III were obtained after pre-concentration of an NPH-CSF sample by a factor of approx. 10. Applying the same procedure (second IP-round with mAb 14-2-4) to an independent, 10-fold concentrated NPH-CSF sample revealed the mass spectrum in B. Linear mode mass spectra are shown with annotations of the respective amyloid-β (Aβ) peptides on top. The signals for Aβ−3−40 are highlighted in red. Aβ variants were assigned to the respective signals only when confirmed by accurate mass information from reflector mode mass spectra (not shown).
Supplementary Figure 4. Monoclonal antibody 14-2-4 does not show appreciable cross reactivity with Aβ1-40 on a sandwich type electrochemiluminescence immunoassay at a concentration of up to 1500 pg/mL. A small spot streptavidin assay plate (Mesoscale Discovery) was blocked and coated with biotinylated mAb 14-2-4 (anti amyloid-β (Aβ)−3–x) (approx. 0.5 µg/mL) essentially as previously described for mAb 101-1-1 (Klafki et al. 2020). A 4-fold dilution series of synthetic Aβ−3–40 in Diluent-35 ranging from 1500 pg/mL to 0.37 pg/mL and, for comparison, Aβ1-40 at concentrations of 1500 and 375 pg/mL were added in duplicates and incubated for 1 hour at room temperature on a mixer. After three washes with MSD wash buffer (MSD), the SULFO-TAG Aβ40 detection antibody (MSD) was added for another hour at room temperature with mixing. The plate was washed three times with wash buffer before 2 x read buffer (25 µL per well) was added and the signal recorded on a SQ120 Quickplex reader. Except for the capture antibody, the assay protocol was essentially identical to the one we have published before with mAb 101-1-1 (Klafki, H. W. et al. (2020) Development and Technical Validation of an Immunoassay for the Detection of APP669-711 (Aβ−3–40) in Biological Samples. Int J Mol Sci, 21, 6564).
Supplementary Table 1: Shapiro-Wilk tests for normal distribution of selected biomarker candidates

|                | Amyloid PET-negative | Amyloid PET-positive |
|----------------|----------------------|----------------------|
| Aβ−3–40        | 0.0352               | 0.2425               |
| Aβ−3–40/Aβ42   | 0.3335               | 0.6452               |
| Aβ42/Aβ−3–40   | 0.1571               | 0.022                |

*Shapiro-Wilk test for normality, α = 0.05