Supplementary Online Content


eAppendix. Materials and methods
eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix: Materials and Methods

CSF ELISA

CSF total tau (T-tau) was determined using a sandwich ELISA (INNOTEST®hTAU-Ag, Innogenetics, Gent, Belgium) specifically constructed to measure all tau isoforms irrespectively of phosphorylation status, as previously described.\(^1\)

Tau phosphorylated at threonine 181 (p-tau181) was measured using a sandwich ELISA method (INNOTEST® PHOSPHO-TAU(181P), Innogenetics, Ghent, Belgium), as described previously in detail.\(^2\) Aβ1-42 levels were determined using a sandwich ELISA (INNOTEST® β-AMYLOID(1-42), Innogenetics, Gent, Belgium), specifically constructed to measure Aβ containing both the first and 42\(^{nd}\) amino acid, as previously described.\(^3\)

Immunoprecipitations and immunoblots

To assay Aβ*56, we immunoprecipitated CSF Aβ species using 6E10 (Covance, Dedham, MA), a monoclonal antibody recognizing the N-terminus of Aβ, and detected the captured species on western blots using biotinylated 6E10 (Covance, Dedham, MA). Each coded specimen contained 750 μl CSF, and 240 μl aliquots of each sample were assayed in triplicate and the results averaged. The code was provided after the assays were completed. Briefly, we depleted CSF of endogenous immunoglobulins with Protein-G Fast Flow Sepharose beads (GE Healthcare, Piscataway, NJ) and then immunoprecipitated sAPP\(\alpha\) and Aβ species with 6E10 using Protein-G coated magnetic beads (Life Technologies, Grand Island, NY). The beads were washed twice with 0.1% Triton-X100 buffer, and proteins were eluted by boiling in 30μl SDS-PAGE loading buffer. Eluted proteins were size-fractionated on 10.5-14% Tris-HCl precast gels (Biorad, Hercules, CA), which optimized separation of high molecular weight (>40 kDa) proteins, but
prevented distinguishing Aβ monomers from dimers. The proteins were electro-transferred onto 0.2μm nitrocellulose membranes for 3h. Membranes were boiled in phosphate buffered saline for 25s and blocked with 5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) in 0.1% TBS-T (Tris Buffered Saline –Tween 20) for 1h at room temperature. Membranes were probed with biotinylated 6E10 (1:2500) followed by NeutrAvidin-HRP(1:5000) (Life Technologies, Grand Island, NY). Membranes were then developed using West Pico electrochemiluminescence (ECL) detection system (Thermo Scientific, Rockford, IL) and bands were quantified in arbitrary units (A.U.) using Optiquant software (Packard Instruments, Meriden, CT).

eReferences

