Supporting Information

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SI Materials and Methods

Cell Culture Conditions and Transfections. All experiments were performed in several different cell lines, some of which required transient transfection of apolipoprotein E (ApoE) isoforms alone and some that required transfections of amyloid precursor protein (APP) and ApoE isoforms. Cell lines included HN33 (mouse hippocampal neuron × N18TG2 neuroblastoma), HEK-293T, SH-SY5Y, and H4 human neuroglioma cells that required transient transfection of APP and ApoE isoforms and A172 human glioblastoma cells that expresses APP endogenously that required transient transfection of only ApoE isoforms. Cells were cultured in DMEM containing 10% (vol/vol) FBS and 1% (vol/vol) penicillin/streptomycin. pcDNA3-APP695 was as described previously (1). Human ApoE cDNA constructs were generously provided by Yadong Huang (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco). The PCR product encoding modified ApoE4Δ (amino acids 1–272) was subcloned into a pFLAG-CMV-3 vector (Sigma) containing an amino-terminal FLAG fusion peptide with the protein expression being driven by the human cytomegalovirus immediate-early promoter (2–4). All DNA constructs were confirmed by sequence analysis.

Transient transfection of cells with Lipofectamine 2000 (Invitrogen) or FuGENE-6 or X-tremeGENE (both from Roche) was performed as described earlier (5, 6) or according to the manufacturer's instructions. The transfection efficiency using these conditions was about 50–70%. Cells were transfected with ApoE alone or APP/ApoE constructs.

Cell Extracts, Immunoprecipitation, and Western Blotting. Following transfection of cells with APP and ApoE isoforms or ApoE isoforms alone, culture media and cell extracts were collected. Whole cell extracts were prepared as described earlier (6, 7). Briefly, cells were collected and resuspended in cell lysis buffer (500 mM Hepes, pH 7.6, 250 mM NaCl, 0.1% Nonidet P-40, 5mM EDTA) containing complete miniprotease inhibitors (Roche). Resuspended cells were sonicated three times for 30 s each, and unbroken cells and membrane debris were removed by centrifugation at 4° for 15 min at 20,000 × *g*. Typically, 100 μg of cell extract was loaded for SDS/PAGE and Western blot analysis as previously described (6, 7). Culture media and cell extracts (150–200 μg protein) were subjected to immunoprecipitation (IP) with an N-terminal anti-APP antibody that recognizes soluble amyloid precursor protein alpha (sAPPα) or 5A3/1G7 mixed monoclonal antibody (recognizing the ectodomain of APP) or anti–CT-15-APP antibody. The immunoprecipitated proteins were subjected to SDS/PAGE and Western blotting (6, 7). Enhanced chemiluminescence detection of the proteins was performed with Pierce ECL detection reagent (Thermo Scientific).

Antibodies. The following APP antibodies were used in the present studies: 5A3/1G7 mixed monoclonal antibody (recognizing the ectodomain of APP) (8, 9); APP-CT15 polyclonal antibody (both kindly provided by Edward Koo, University of California, San Diego); APP-6E10 monoclonal antibody (Covance); and N-terminal APP polyclonal antibody (Sigma). Other antibodies included ApoE antibody (Sigma), APP-phosphoThr668 antibody (Cell Signaling), Tau and phosphorylated (p)-Tau antibodies (Invitrogen and Cell Signaling), and SirT antibodies (Cell Signaling). All HRP-conjugated secondary antibodies were purchased from Amersham/GE Healthcare or Santa Cruz Biotechnology.

sAPPα, sAPPβ, and Aβ Assay. sAPPα or sAPPβ secreted into the cellular media was determined with the AlphaLISA sAPPα and sAPPβ immunoassay research kits (Perkin Elmer) according to the manufacturer's protocol with some modifications (10, 11). The standards, blanks, and media were diluted with the buffer provided in the kit and added to the plate. During the first incubation step, the analyte was captured either by an antibody recognizing the α-secretase cleavage site at sAPPα C terminus (clone 2B3) or the sAPPβ C terminus and by a second biotin-labeled antibody specific to the N-terminal part of sAPP (common for both sAPPα and sAPPβ). In the second incubation step, the biotinylated anti-analyte antibody was bound to the streptavidin-coated donor beads. At the end of this reaction, the plates were read on an EnSpire Alpha 2390 multilabel plate reader equipped with the AlphaScreen module. The Aβ1-42 or 1-40 was determined from media or cells using Invitrogen's sandwich ELISA kit as described previously (10–12). The levels of Aβ were quantified from a standard curve and normalized to total cellular protein.

Quantitative Real-Time PCR. To analyze the mRNA levels of Sirtuin T1 (SirT1), SirtT2, and SirT6, real-time PCR was performed on first-strand cDNAs. Primers for SirT1, SirT2, and SirT6 were designed using the Roche universal probe library system; a custom assay and the primers were synthesized by Integrated DNA Technology. Real-time PCR was performed in SYBR Green master mix (Roche) with the corresponding primer sets. A172 cells were transfected with ApoE3 or ApoE4 cDNA, and after 24 h, the cells were trypsinized and collected. Total RNA was isolated using High Pure RNA isolation kit (Roche). RNA concentrations were measured using the NanoDrop ND-1000 spectrophotometer, and 1 μg of RNA from untransfected and each transfected sample was reverse transcribed. The real-time PCR cycling was performed on the Light Cycler 480 (Roche) using white Light Cycler480 384-multiwell plates. The melting curves of PCR products were monitored to ensure that a single melting curve was obtained. For analysis of the real-time PCR data, δCt values of samples were normalized to values obtained for GAPDH, which was assayed simultaneously. Relative quantification using the Delta Delta Ct method was adopted to calculate the relative quantity of SirT1, SirT2, and SirT6 levels.

Restriction Isotyping of Human ApoE. We used restriction enzyme isoform genotyping to identify the ApoE alleles as described previously (13, 14). The method involved using primer pairs to amplify the ApoE gene sequence containing amino acid positions 112 and 158. The amplification products were later digested with HhaI and subjected to electrophoresis on polyacrylamide gels. Each of the isoforms was distinguished by a unique combination of HhaI fragment sizes (Fig. S5) that enabled explicit typing of all homozygotic and heterozygotic combinations.

Human Autopsy Material. Dissected frozen tissues representing different regions of the brain were provided by Stephen DeArmond (School of Medicine, University of California, San Francisco), and research was conducted in compliance with policies and principles contained in the Federal Policy for the Protection of Human Subjects. Eleven postmortem human brains [five normal and six with Alzheimer's disease (AD)] were used in this study. Limited information (sex, age at death, and pathology status) that was available only on a subset of samples is provided in Table S2. Restriction enzyme isoform genotyping to identify the ApoE

alleles of each sample was performed as described above. Samples for SDS/PAGE and Western blots were prepared as described earlier (15). Samples (100 μg) were added to reducing sample buffer and heated for 10 min at 70 °C before subjecting them to SDS/PAGE.

Surface Plasmon Resonance. Thioredoxin (trx)-fusion proteins containing ApoE3 (trx-ApoE3) and ApoE4 (trx-ApoE4) was expressed and purified as described previously (16). In addition, four fusion proteins containing different fragments of APP were expressed and purified as described previously (17). *Escherichia coli* thioredoxin (Sigma Chemicals) was purified using size-exclusion chromatography. Proteins were biotinylated using the EZ-Link Sulfo-NHS-SS-Biotin Biotinylation Kit (Thermo Scientific) following the instructions to produce one to two biotins per molecule.

For the ApoE-binding experiments, avidin (Thermo Scientific) was immobilized on a CM5 biacore chip via a standard amine coupling protocol (Biacore Handbook). Biotinylated proteins were loaded onto the chips to create two chips: one with an avidin-only reference channel (FC1), thioredoxin (FC2), ApoE3 (FC3), and ApoE4 (FC4) and one chip with an avidin-only reference channel (FC1), MBP-eAPP$_{19–624}$ (FC2, FC3), and eAPP$_{230–624}$ (FC4). Nonbiotinylated proteins were also dialyzed against 20 mM phosphate, pH 6.5, 125 mM NaCl, and 0.005% Tween-20 (PBST) and then concentrated to between 0.5 and 2.0 mg/mL The last dialysis buffer was filtered with a 22-μM filter and used as running buffer in the surface plasmon resonance (SPR) experiments. The maximal saturation for each concentration of protein was estimated by modeling the association phase with either a single phase association or two phase association model in PRISM (Graphpad). The $K_{D,\ eff}$ was calculated using the predicted plateaus for each concentration and a single-binding site model.

For the competition experiment, varying concentrations of disulfiram were incubated with either 0.15 or 0.075 μM ApoE4 in 20 mM phosphate, pH 6.5, 125 mM NaCl, and 1% DMSO before being pumped through the chip. For disulfiram-APP binding experiments, Trx-eAPP$_{575–624}$ or thioredoxin was coupled to the CM5 biacore chips using standard amine coupling techniques. The disulfiram was diluted from a 10 mM solution in DMSO to 50 μM in 1% DMSO, 20 mM sodium phosphate, pH 6.5, 125 mM sodium chloride, and 0.005% Tween and then serially diluted by 1.5 for 10 steps.

Statistical Analysis. Statistical significance for all of the tests assessed by calculating the P values and defined as $P < 0.05$ was calculated by one-way ANOVA followed by Newman-Keuls multiple comparisons post hoc testing.

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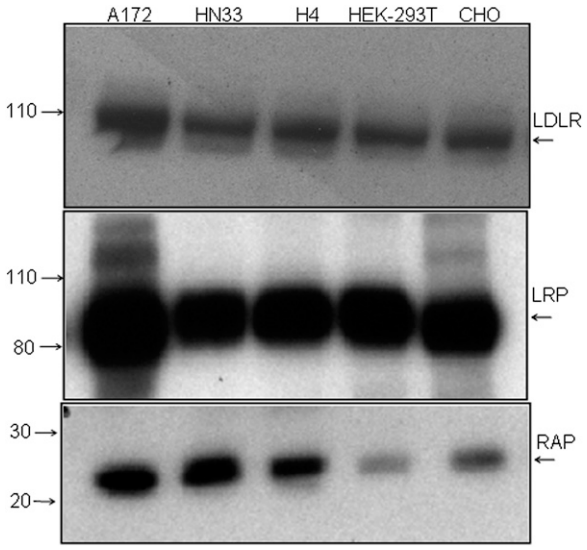

Fig. S1. Expression profile of ApoE receptors and chaperone proteins. Cell extracts isolated from various cells were subjected to SDS/PAGE and Western blotting for ApoE receptors and specific ER chaperone proteins involved in the uptake of ApoE including low-density lipoprotein (LDLR) receptor (~110 kDa), LDL receptor-like protein (LRP; ~85 kDa), or receptor-associated protein (RAP, ~24 kDa).

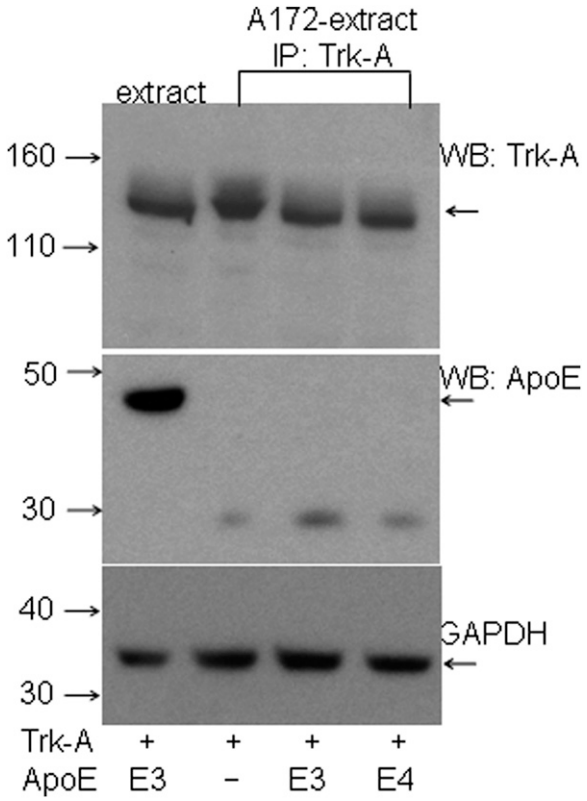

Fig. S2. A172 cells were transfected with TrkA and ApoE isoforms. Twenty-four hours after transfection, cell pellets were collected and lysed, and extracts were prepared. Cell extracts were subjected to immunoprecipitation (IP) with anti-TrkA antibody followed by SDS/PAGE and Western blotting (WB) to detect TrkA or ApoE4 protein expression. The last panel represents endogenous GAPDH (loading control) before the pull-down. Lane 1 represents the extract loaded before the pull down to identify the TrkA and ApoE.

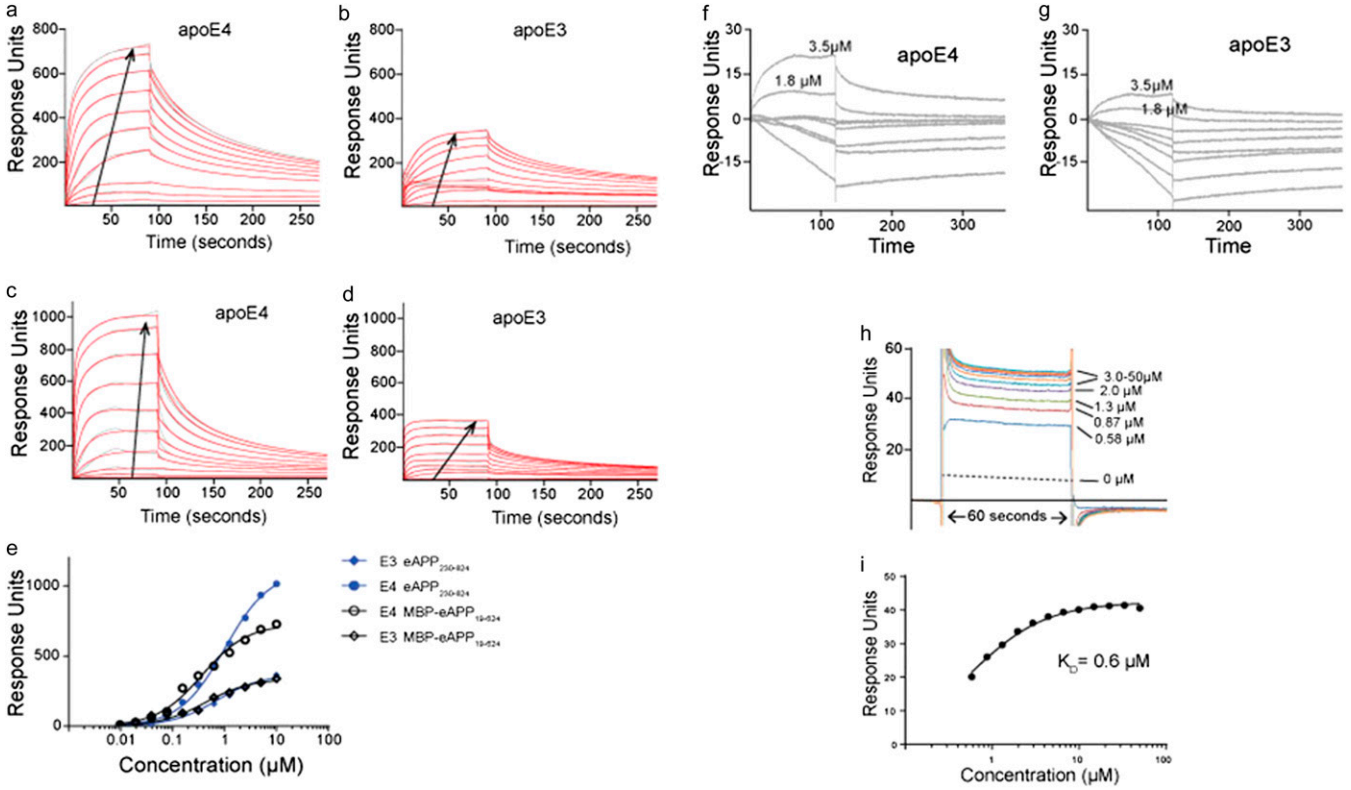

Fig. S3. Binding parameters (*A–I*) derived from surface plasmon resonance for the binding of ApoE3 and ApoE4 for different fragments of the APP ectodomain. The sensograms are shown in gray and the fits are shown in red.

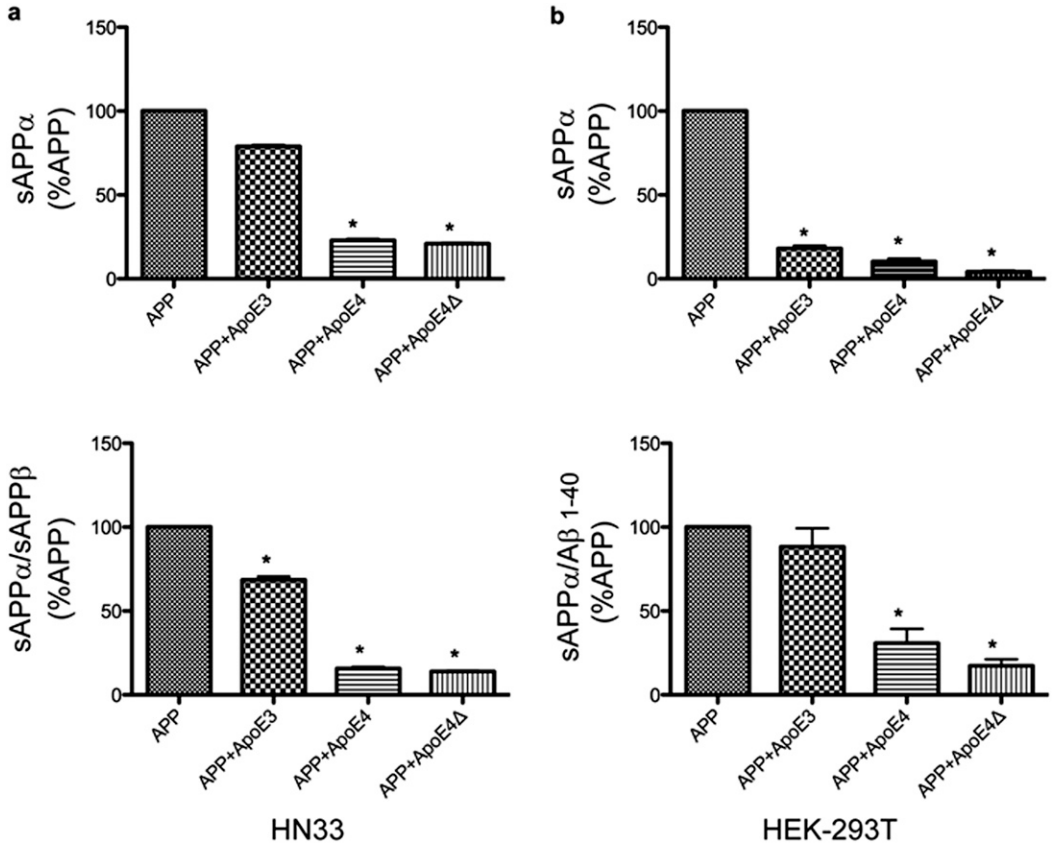

Fig. S4. HN33 (*A*) or HEK-293T cells (*B*) were transfected with APP and ApoE isoforms. Twenty-four hours after transfection, sAPPα and sAPPβ secreted into the medium were determined with the AlphaLISA sAPPα and sAPPβ immunoassay research kit as mentioned in *SI Materials and Methods*. Aβ1-40 in the HEK-293T cell extracts (*B*) was detected with a sandwich ELISA as mentioned in *SI Materials and Methods*. Data (mean ± SE) are from four experiments performed in triplicate, $*P < 0.05$.

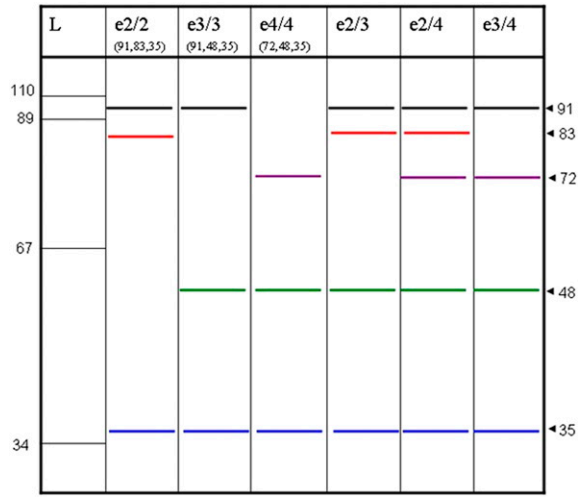

Fig. S5. Restriction genotyping of human ApoE. Isoform genotyping of ApoE was performed as described in *SI Materials and Methods*. DNA isolated from normal and AD brain tissue was subjected to PCR to amplify the ApoE sequence containing amino acid positons 112 and 158. The 244-bp amplified sequence was digested with Hha1 and separated on a polyacylamide gel. The depicted figure is a sketch representing the gel-separated products of ApoE amplification and Hha1 digestion of the DNA. A 35-bp fragment is common to all genotypes. With the exception of this fragment, each genotype revealed unique combinations of Hha1 fragment sizes. Each of the samples from heterozygotic combinations contained both sets of fragments from each ApoE allele.

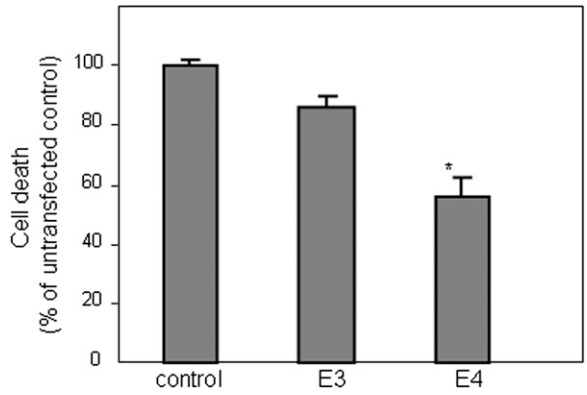

Fig. S6. A172 cells were transfected with ApoE isoforms, and 36 h later, cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, a yellow tetrazole) assay. Surviving and dead cells were counted and data normalized to untreated nontransfected control levels set to 100%. Data (mean ± SE) are from three independent experiments performed in triplicate, $*P < 0.05$.

Table S1. Binding parameters derived from surface plasmon resonance for the binding of ApoE3 and ApoE4 for different fragments of the APP ectodomain

Surface	MW (kDa)	Flow (μL/min)	MW (kDa)	Flow rate (μl/min)	k_D (s^{-1})	$K_{D,\ eff}$ (nm)
TRX-apoE4	45*	TRX-eAPP$_{575–624}$	20	45	ND	>>3,500
		TRX-eAPP$_{290–624}$	52	45	0.007	80 ± 20
TRX-apoE3	45*	TRX-eAPP$_{575–624}$	20	45	NS	NS
		TRX-eAPP$_{290–624}$	52	45	0.006	300 ± 50
TRX	10	TRX-eAPP$_{575–624}$	20	45	NS	NS
		TRX-eAPP$_{290–624}$	52	45	NS	NS
MBP-eAPP$_{19–624}$	127	TRX-apoE4	180†	60	0.009	370 ± 40
		TRX-apoE3	180†	60	0.010	430 ± 80
eAPP$_{230–624}$	45	TRX-apoE4	180†	60	0.011	720 ± 120
		TRX-apoE3	180†	60	0.010	1,000 ± 67

The sensograms corresponding to the table entries are shown in Fig. 2 and Fig. S3. ND, not determined; NS, not signficant binding.

*Molecular weight of the monomer.

†Molecular weight estimated by size-exclusion chromatography. This molecular weight is consistent with previous findings that nonlipidated apoE being a tetramer in solution.

Table S2. Dissected frozen tissues representing different regions of the brain obtained from the Neuropathology Division, University of California, San Francisco

Lane number	Patient sample number/sex	Age (y)	ApoE profile	Pathology
1	P-1065/M	28	E2/E4	Non-AD control-hepatic encephalopathy
2	P-1254	NA	E2/E3	Non-AD control
3	P-1302/F	58	E3/E3	Non-AD control-diagnosed with frontotemporal dementia and striatonigral degeneration
4	P-1462	NA	E3/E4	Non-AD control
5	P-1640	NA	E3/E4	Non-AD control
6	P-1120	NA	E3/E4	Alzheimer's disease
7	P-1158	NA	E2/E4	Alzheimer's disease
8	P-1171/M	83	E2/E4	Alzheimer's disease
9	P-1476/F	76	E2/E4	Alzheimer's disease
10	P-1516/M	82	E3/E3	Alzheimer's disease + diffuse Lewy body disease
11	P-1565/M	64	E3/E4	Alzheimer's disease

F, female; M, male; NA, information not available.