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# Supplementary Information for

## α-synuclein-lipoprotein interactions and elevated ApoE level in Parkinson's disease patients CSF

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References for SI reference citations

**Supplementary Materials and Methods**

***Subjects and sample collections:*** All patients fulfilled the clinical diagnosis criteria for Parkinson's disease [2], MSA [5] and PSP [7] respectively. The disease severity of the Parkinson's disease patients was scored with the Unified Parkinson's disease rating scale (UPDRS) and Hoehn&Yahr scale. Medications against Parkinson's disease are summarized as L-dopa equivalent doses (LEDD) [10]. Additionally, the Montreal Cognitive Assessment (MoCA), Beck's Depression Inventory (BDI) and Non-motor symptoms questionnaire (NMSQ) scores were obtained. Control subjects were healthy volunteers or had mild symptoms without any severe neurological diagnosis (e.g. temporary tension headache or sensory symptoms). Controls were matched to Parkinson's disease cases according to age and gender (Table. 1). The standardized lumbar puncture procedure was performed sitting up, in accordance with the Alzheimer's Disease Neuroimaging Initiative recommended protocol. Samples were collected into sterile polypropylene tubes, the first 2mL were discarded and between 10 and 12mL CSF from the first portion was collected and gently mixed in order to minimize the gradient influence. Cell counts were measured and samples were centrifuged in the original tube at 4000rpm for 10min at 4°C. CSF samples were aliquoted, frozen on dry ice and stored at –80°C until assayed. Time between sample collection and freezing was maximum 30min. Blood contaminated samples were excluded (erythrocyte count > 10cells/mL). Formalin-fixed, paraffin-embedded 5µm human brain sections, from subjects without neurological disorders (n=2; male, age 77 and male, age 73) or Parkinson's disease (n=2; male, age 78, disease duration 12 years and male, age 75, disease duration 12 years), were obtained from the Brain Bank at Karolinska Institutet.

***Co-Immunoprecipitation (co-IP):*** A large volume of CSF (50mL) was pulled down from healthy controls. Next, the sample was mixed with PBS buffer (20mM phosphate, 150mM NaCl, pH-7.4) containing 5 mM 2,2',2",2"'-(Ethane-1,2-diyldinitrilo)tetraacetic acid (EDTA) and protease inhibitors in 1:9(v/v) ratio. All incubation steps were performed upon gentle agitation. A/G agarose beads were pre-treated for 1h with 1% bovine serum albumin (BSA), dissolved in PBS buffer, to block any unspecific interactions. The endogenous immunoglobulins were depleted from the sample during 2h incubation with protein A/G agarose beads (Abcam) at room temperature (RT), followed by elimination of beads via centrifugation (14.000xg, 4°C, 10min). The antibody recognizing αSN, used for similar approaches previously [3, 6, 8], was added 1:100(v/v) to the obtained solution and incubated overnight in 4°C. Thereafter, the sample was incubated for 6h with protein A/G agarose beads at RT and spun (3000xg for 2min at 4°C). The supernatant was removed and beads were washed three times with PBS containing 5mM EDTA and protease inhibitors. The protein elution was conducted by 0.2M glycine pH-2.6 (1:1 initial beads volume to glycine volume) during 10min incubation. The elution step was repeated two more times, all elutes were pooled together and neutralized by adding an equal volume of Tris pH-8.0. Next, the sample was dialyzed against 10mM Tris pH-8.0 and protein concentration was determined using NanoDrop ND-1000 (ThermoFisherScientific). Then, the sample was lyophilized and stored at -20°C for further analysis. In parallel, separate positive and negative control samples were prepared. Recombinant αSN served as a positive control. The negative control was a CSF sample without addition of the

primary anti-αSN antibody. These controls were used to assess any unspecific interactions from A/G agarose beads.

***Mass spectrometry (MS):*** IP samples were dissolved in 6M guanidine HCl, 50mM Tris pH-8.0, 2mM dithiothreitol (DTT), heated at 95°C for 15min, then cooled down and diluted in 50mM $NH_4HCO_3$ (pH-7.8). A modified trypsin (Promega) was then added in a 1:100 protease:protein ratio (w/w) and incubated for 2h at 37°C. Proteolysis was stopped by quick cooling in dry ice. Obtained peptides were purified using C18 resin ZipTips (Millipore). Briefly, tips were activated with 70% acetonitrile (ACN), 0.1% trifluoreacetic acid (TFA) and washed three times with 0.1% TFA. Next, the sample was loaded onto resin by gently pipetting back and forth through a tip 20 times, putting flow through back into the sample tube each time. Resin was washed three times with of 0.1% TFA and bound peptides eluted two times by 70% ACN, 0.1% TFA into a new tube. All samples were dried down to approx. 10µL using a vacuum centrifuge. Thereafter samples were analysed on a nanoLC system (Easy-nLC II, ThermoFisherScientific) coupled to an electrospray linear ion trap (LTQ, ThermoFisher Scientific) mass spectrometer. The sample was injected and desalted on a pre-column (2cm, 100µm I.D, 120Å, 5µm C-18A1; Easy column, ThermoFisher Scientific) at a flow rate of 10µL/min for 10min. A picofrit 15-cm fused silica emitter with a 75µm inner diameter and a 375µm outer diameter (New Objective) was used as the analytical column. The emitter was packed in-house with a slurry of reverse-phased ACE C18-AR 2-µm resin (Scantec Nordic) dispersed in methanol using a pressurized packing devise (Proxeon Biosystems). The mobile phases were BufferA (0.1% Formic acid in water) and BufferB (ACN and 0.1% Formic acid). The peptide samples were separated during 40min gradient from 2-55% BufferB at a flow rate of 250nL/min and the MS data was collected in a data dependent manner. The acquisition continuously switches between full MS scan (m/z 300-2000), zoom scan (most intense peak in full scan) and MS/MS scan (most intense peak in zoom scan) where the most intense peak can be picked twice in a time window of 40s and is then put on an exclusion list during a 150s period. The MS/MS data was converted into a combined mgf-file. The mgf-files were searched against *H. sapiens* database using a X!Tandem search engine. The following settings were used for the database search: peptide mass tolerance of ±0.5Da; fragment mass tolerance of ±0.5Da; Carbamidometyl Cys, or unspecific cleavage, possible post-translational modifications (N-terminal acetylation, N-terminal pyro-glutamic acid derived from glutamine, C-terminal amidation, oxidation of methionine and tryptophan, phosphorylation of serine, tyrosine and threonine, deamidation of asparagine and glutamine). A significance threshold of log<-2 was used for individual peptide.

***Immunogold labelling and Transmission Electron Microscopy (TEM)*:** To remove small and medium proteins, the CSF sample was initially filtered through a 100kDa Amicon Ultra-15 Centrifugal Filter Unit (Millipore) and the retained solution was collected for TEM analysis. An aliquot of 3µL from each sample was added to a grid with a glow discharged carbon coated supporting film for 3min. The excess solution was soaked off by a filter paper. The anti-ApoE antibody and the anti-αSN antibody, diluted 1:20 each in 0.1M PBS containing 0.1% normal goat serum (PBG), were incubated for 1.5h. Afterwards, grids were rinsed in PBG and bound antibodies were detected using the secondary goat polyclonal anti-mouse (5nm, anti-ApoE antibody detection) or goat polyclonal anti-rabbit (10nm, anti-αSN antibody detection) antibodies at a final dilution of 1:100 each. Next, sections were rinsed in PBS, distilled water and stained with 1% uranyl acetate in

water for 10s and air-dried. The samples were examined in a Hitachi7700 (Tokyo) at 80kV and digital images were taken by a Veleta camera (Olympus).

***Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE):*** To ensure the unbiased data analysis all samples were coded and each single gel include both controls' and patients' samples run randomly. Furthermore, normalization sample, composed of a mixture of several CSF samples for CSF analysis and a mixture of several plasma samples for plasma analysis, was run on each gel. The sample was mixed in 3:1 ratio with a denaturing loading buffer (106mM Tris-HCl, 141mM Tris, 2% lithium dodecyl sulphate (LDS), 10% glycerol, 6% β-mercaptoethanol, 0.51mM EDTA, 0.22mM SERVA Blue G-250, 0.175mM Phenol Red, pH-8.5) and boiled at 95ºC for 5min. Next, samples were separated using a bis-tris acrylamide gel using MES running buffer (50mM Tris, 50mM 2-(N-morpholino)ethanesulfonic acid (MES), 0.1% SDS, 1mM EDTA, pH-7.3). 20µL and 5µL of sample was used for accordingly CSF and plasma analysis. If necessary, the gel was stained using Coomassie Brilliant Blue (CBB) R-250 solution (0.1% CBB R-250/40% methanol/10% glacial acetic acid) and distained using 4%/4% glacial acetic acid/ethanol solution. SDS-PAGE gels were scanned using standard table-top scanner or red channel on Odyssey™ CLx Imaging System (LI-COR). The intensity of protein bands was analysed using ImageJ[1].

***Western blotting (WB):*** After SDS-PAGE, gels were incubated in a transfer buffer (25mM Tris, 192mM glycine, 30% methanol) and 0.45µm pore size Immobilon-P™ PVDF membranes (Millipore) were pre-wetted in methanol. Gels were then assembled with membranes using TE22™ Mini Tank Transfer Unit cassettes (GE Healthcare) and overnight transfer was performed at 4°C. Next, membranes were dried and shortly fixed in 100% methanol. For detection of small proteins, in samples with a low total protein content, a mixture of 3% paraformaldehyde/0.01% glutaraldehyde in PBS was used as a fixative [9]. Between each incubation period, membranes were washed three times in Tris Buffered Saline (TBS, 20mM Tris, 150mM NaCl, pH-7.6) containing 0.1%(v/v) Tween20 (TBS-T). Membranes were blocked during 1h incubation in 5% skim milk at RT. Next, membranes were incubated overnight at 4°C with a primary antibody diluted 1:2000(v/v) in 1% skim milk. Membranes were thereafter incubated for 2h with appropriate HRP-conjugated secondary antibody (Dako) diluted 1:10000(v/v) in 1% skim milk at RT. The signal was developed by a Clarity™ Western ECL Substrate (Bio-Rad), specificity of antibodies was checked (Fig. S1) and levels of visualized proteins were estimated by measuring band intensities with ImageJ [1]. When necessary a colorimetric scan was used to visualize the protein ladder. Additionally, since there is no loading control available for the CSF analysis and the total protein content may change due to many factors, correlation analyses between apolipoproteins' levels and both albumin and IgG were performed to ensure that their levels are not changing with the total protein content. Moreover, on all blots from the same cohort, normalization sample, composed of the mixture of several CSF or plasma samples, was used to compare results across blots.

***Immunohistochemistry (IHC):*** Human formalin-fixed, paraffin-embedded 5µm section from subjects without neurological disorder (n=2; male, age 77 and male, age 73) or Parkinson's disease (n=2; male, age 78, disease duration 12 years and male, age 75, disease duration 12 years), were obtained from the Brain Bank at Karolinska Institutet. Sections were deparaffinised, rehydrated and a heat-induced epitope retrieval was done in Tris-EDTA buffer (10mM Tris base, 1mM EDTA solution, 0.05% Tween20, pH-9.0) for 20min at 95°C followed by a 10min rinse in cold water.

Sections were washed 4 times in PBS between each incubation period. The endogenous peroxidase activity was quenched for 15min in a mix of 3% hydrogen peroxide and 10% methanol in PBS. One-hour incubation in 5% goat normal serum proceeded overnight incubation with the anti-ApoE antibody in 2.5% goat normal serum at 4°C. Thereafter, sections were incubated with the HRP conjugated antibody in 1% goat normal serum. The peroxidase labelling was visualized with mix of 3,3–diaminobenzidine and nickel (SK-4100, Vector Laboratories) which yielded a blue/grey reaction product. A negative control, omitting the primary antibody, was performed in parallel.

***Preparation of recombinant αSN monomers, oligomers and fibrils:*** αSN plasmid vector pET11-D, containing the insert coding human αSN, was expressed in *E.coli* BL21(DE3) competent cells using an auto-induction method. Cells were harvested by centrifugation and treated with the osmotic shock buffer (20mM Tris-HCl, pH-7.2, 40% sucrose), incubated for 10 min and centrifuged again. Afterwards, the pellet was suspended in ice-cold deionised water, with subsequent addition of saturated $MgCl_2$, and briefly incubated on ice. The periplasmic fraction of the cell lysate was collected and majority of unwanted proteins precipitated by adjusting pH to 3.5 with 1M HCl. Soluble proteins were collected by centrifugation and pH of the obtained supernatant was adjusted to pH-7.5 with 1M NaOH. The solution was filtered and fractionated on a Q-Sepharose column connected to an ÄKTA Explorer system (GE Healthcare) using rising concentration of NaCl. Fractions containing αSN were identified by SDS-PAGE and pulled together. Further, high molecular weight aggregates were removed by filtration through a 30kDa cut-off filter, to obtain only monomeric form of αSN, and analysed with SDS-PAGE to ensure purity. Final solution was dialyzed against deionised water. The αSN concentration was determined using NanoDrop ND-1000 (Thermo Scientific), protein was aliquoted, lyophilized and stored at -20°C.

To obtain αSN oligomers, αSN monomers ware dissolved to 12 mg/ml and incubated in an eppendorf shaker at 37°C, 900 rpm for 5 hrs. Insoluble material was removed by centrifugation at room temperature at 16000 x g for 10 min. The supernatant were loaded on Superpose 6 column (GE Healthcare) connected to an ÄKTA Explorer system (GE Healthcare) in PBS buffer at a flow-rates of 2 ml/min. Oligomer fractions were collected, concentrated with a 50kDa cut-off spin filter and stored at 4 °C.

The recombinant αSN was fibrillated as described before [4]**.** αSN monomers were dissolved at 1 mg/ml and incubated at 37°C with shaking for 5 days. Obtained samples was centrifuged at room temperature at 16000 x g for 10 min and obtained pellet was suspended in PBS buffer. To obtain unified length on fibrils (pre-formed fibrils, PFF) each sample was briefly sonicated.

For aggregation analysis the recombinant αSN, with or without addition of ApoE, was fibrillated at a final concentration of 1mg/mL for αSN and 0.25mg/mL for ApoE with 40μM ThT in a Tecan Spark 10M (Tecan Nordic AB) plate reader at 37°C with 5min shaking and signal readout every 10min. The ThT signal was monitored at 448nm excitation and 485nm emission.

**Supplementary Figures and Tables**

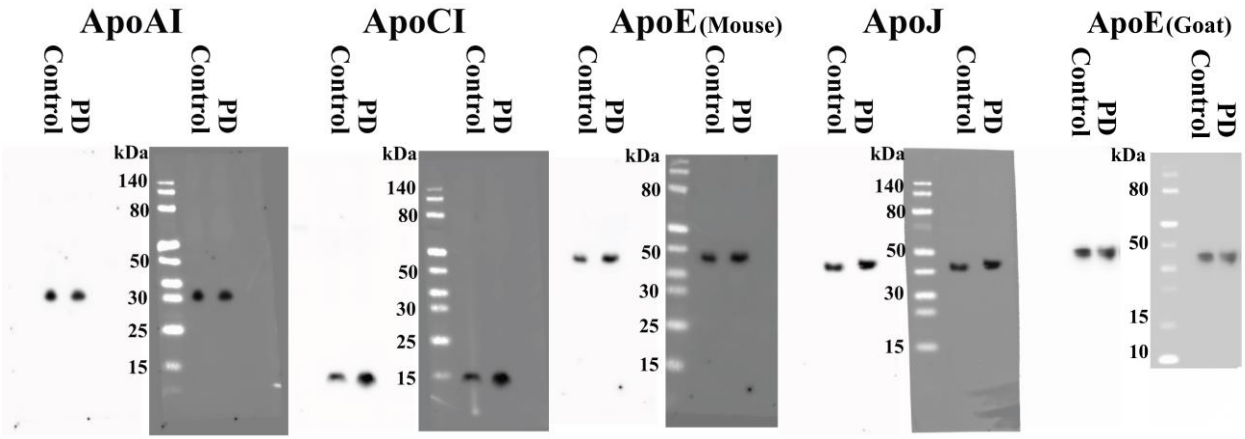


**Fig. S1. Anti-apolipoproteins antibodies specificity using the WB method.** Representative autoradiograms (left) and autoradiograms merged with colorimetric scans of the membrane (right) from films obtained after running CSF samples and performing WB procedure against analysed apolipoproteins.

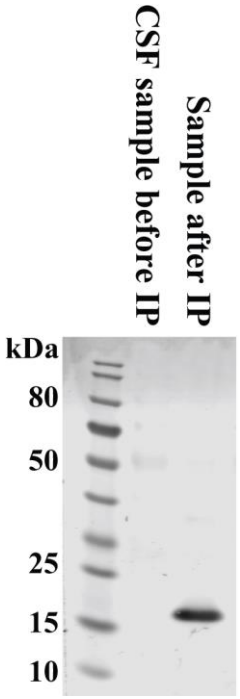


**Fig. S2. Detection of αSN before and after Immunoprecipitation from CSF.** An autoradiogram of the WB membrane performed against αSN on the CSF sample before (left) and after IP against αSN (right). No signal is observed for the CSF sample before IP, due to a very low concentration of αSN in the crude CSF. Antibody used – the rabbit polyclonal anti-αSN antibody (sc-7011-R, Santa Cruz).

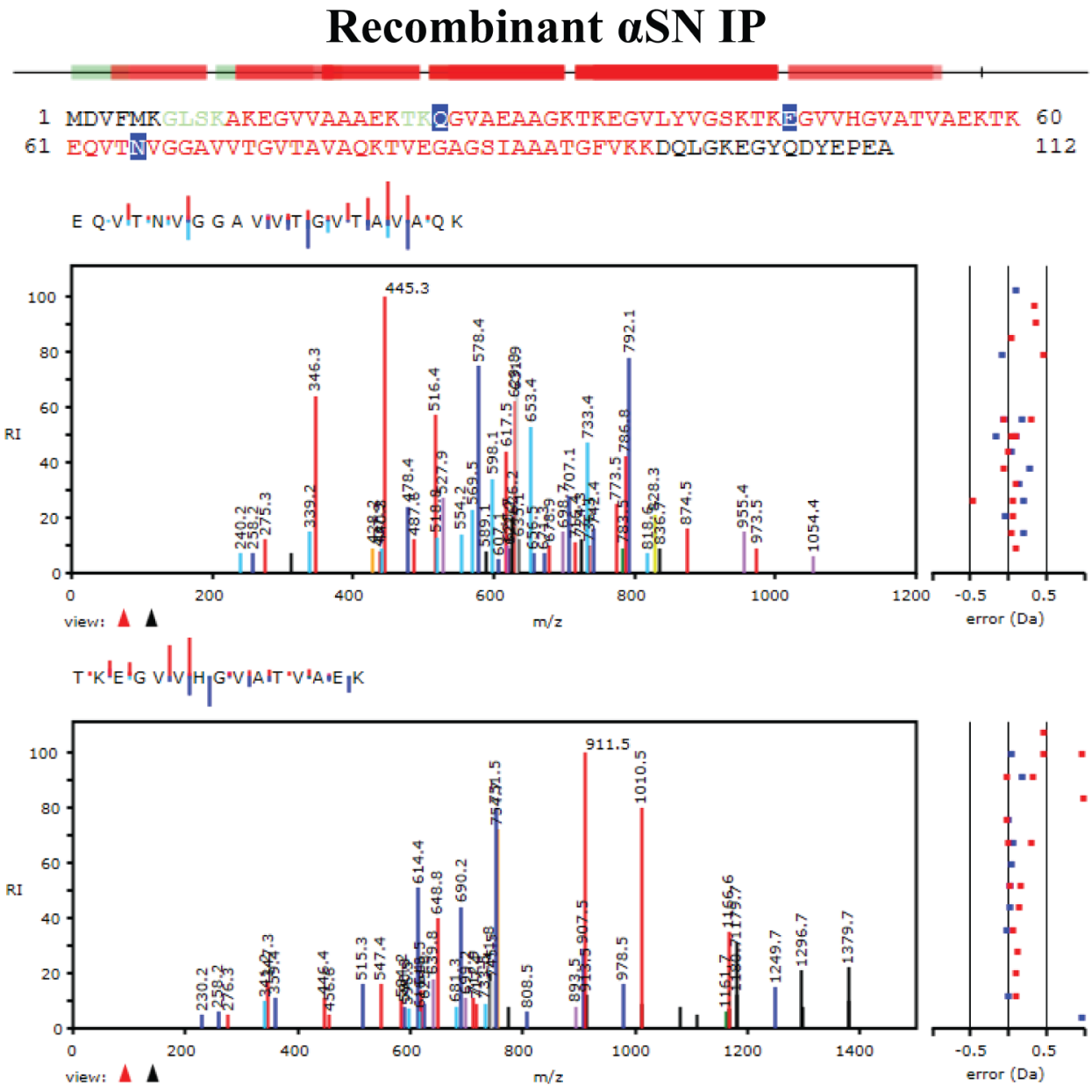


**Fig. S3. The αSN sequence coverage, and two selected peptide spectra, obtained after the trypsin cleaved peptides fragmentation for samples of the recombinant αSN after IP**. Red – residues from a peptide domain observed at least once during the analysis; Green – residues predicted to be difficult to observe by standard techniques; Blue – residues found are chemically modified.

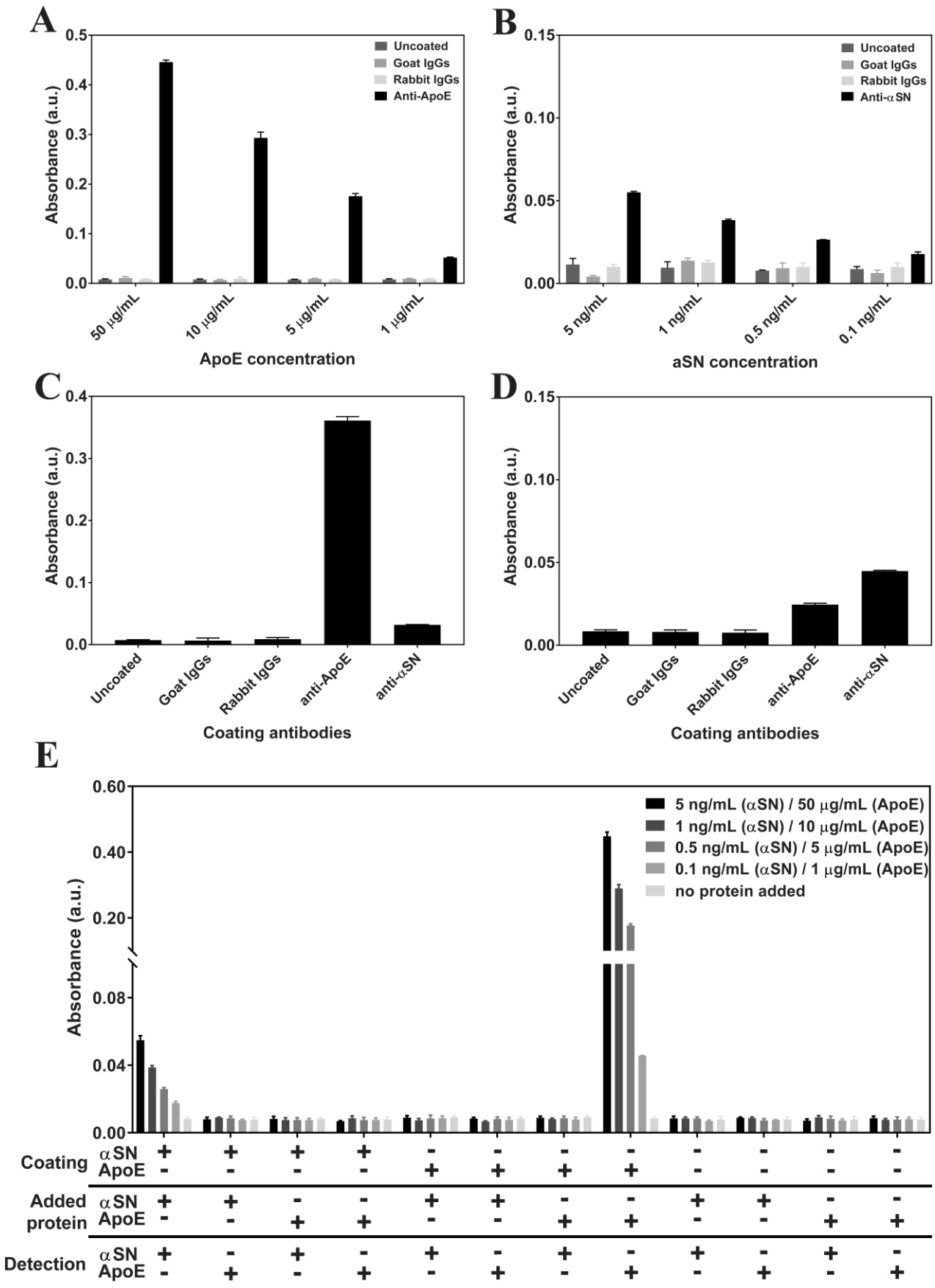
A
Absorbance (a.u.)
0.5
0.4
0.3
0.2
0.1
0.0
Uncoated
Goat IgGs
Rabbit IgGs
Anti-ApoE
50 μg/mL
10 μg/mL
5 μg/mL
1 μg/mL
ApoE concentration
B
Absorbance (a.u.)
0.15
0.10
0.05
0.00
Uncoated
Goat IgGs
Rabbit IgGs
Anti-αSN
5 ng/mL
1 ng/mL
0.5 ng/mL
0.1 ng/mL
aSN concentration
C
Absorbance (a.u.)
0.4
0.3
0.2
0.1
0.0
Uncoated
Goat IgGs
Rabbit IgGs
anti-ApoE
anti-αSN
Coating antibodies
D
Absorbance (a.u.)
0.15
0.10
0.05
0.00
Uncoated
Goat IgGs
Rabbit IgGs
anti-ApoE
anti-αSN
Coating antibodies
E
Absorbance (a.u.)
0.60
0.40
0.20
0.08
0.04
0.00
5 ng/mL (αSN) / 50 μg/mL (ApoE)
1 ng/mL (αSN) / 10 μg/mL (ApoE)
0.5 ng/mL (αSN) / 5 μg/mL (ApoE)
0.1 ng/mL (αSN) / 1 μg/mL (ApoE)
no protein added
Coating
αSN + + + + - - - - - - - -
ApoE - - - - + + + + - - - -
Added protein
αSN + + - - + + - - + + - -
ApoE - - + + - - + + - - + +
Detection
αSN + - + - + - + - + - + -
ApoE - + - + - + - + - + - +

**Fig. S4. Specificity of antibodies used for Immunoprecipitation and ELISA.** (A) Detection of the recombinant ApoE on: an uncoated ELISA plate, coated with goat IgGs, rabbit IgGs or the goat polyclonal anti-ApoE antibody (178479, EMD Millipore). (B) Detection of the recombinant αSN on: an uncoated ELISA plate, coated with goat IgGs, rabbit IgGs or the rabbit polyclonal anti-αSN antibody (sc-7011-R, Santa Cruz). (C) Detection of ApoE in human CSF on: uncoated ELISA plate, coated with goat IgGs, rabbit IgGs, the goat polyclonal anti-ApoE antibody (178479, EMD Millipore) or the rabbit polyclonal anti-αSN antibody (sc-7011-R, Santa Cruz). (D) Detection of αSN in the human CSF on: uncoated ELISA plate, coated with goat IgGs, rabbit IgGs, the goat polyclonal anti-ApoE antibody (178479, EMD Millipore) or the rabbit polyclonal anti-αSN antibody (sc-7011-R, Santa Cruz). (E) Detection of the recombinant ApoE or αSN on: plate coated with the goat polyclonal anti-ApoE antibody (178479, EMD Millipore), the rabbit polyclonal anti-αSN antibody (sc-7011-R, Santa Cruz) and on an uncoated ELISA plate. The detection antibodies used were: the mouse monoclonal anti-ApoE antibody [E6D7] (ab1907, Abcam) and anti-αSN [LB 509] (ab27766, Abcam).

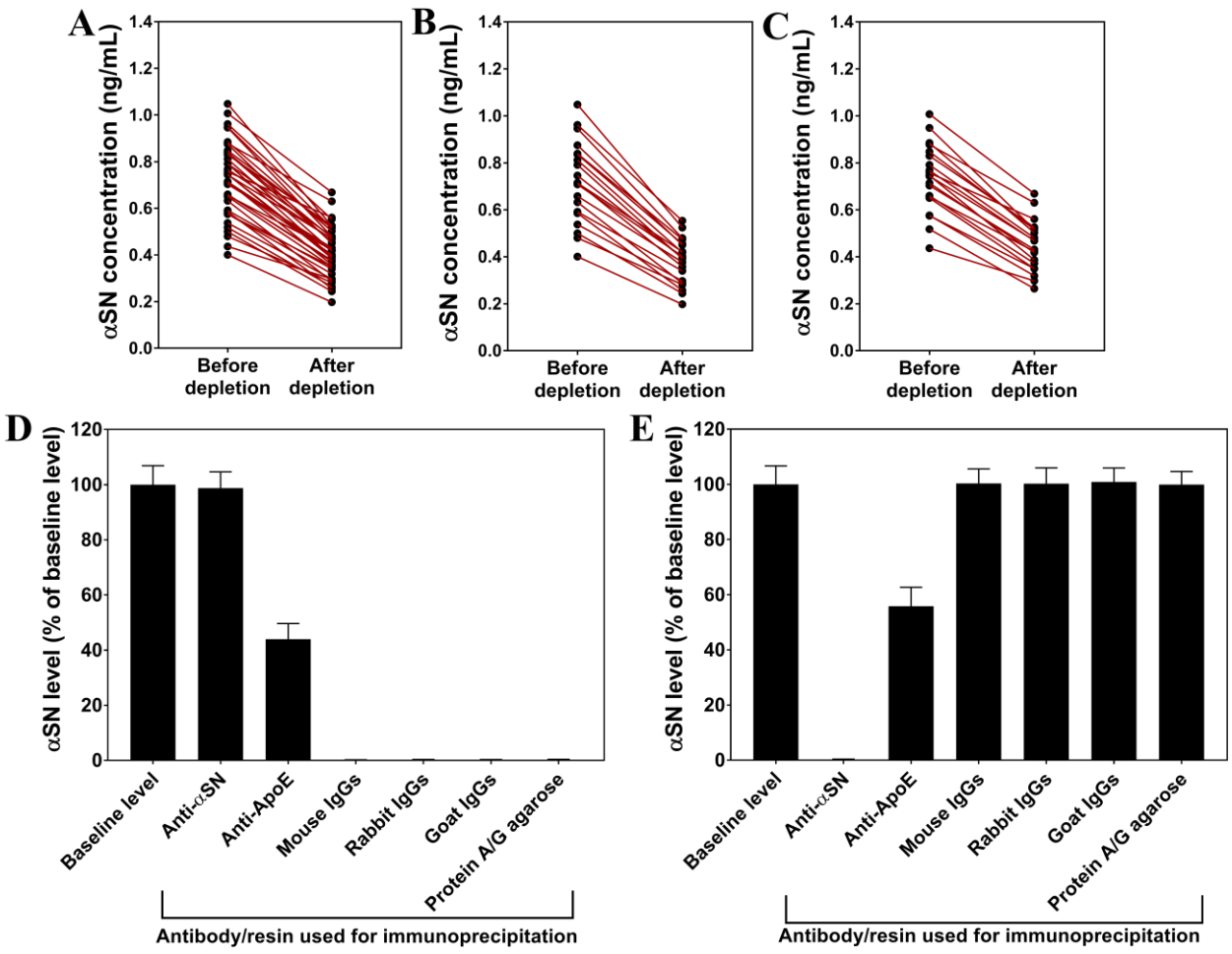


**Fig. S5.** A**nalysis of αSN levels before and after depletion of ApoE-positive vesicles from human CSF samples.** Graph presenting the concentrations of αSN in 40 CSF samples before and after the depletion of ApoE-positive vesicles (A). Depletion of ApoE-positive vesicles uniformly reduced αSN levels in both Parkinson's disease cases (n=20) (B) and healthy controls (n=20) (C). (D) Detection of baseline level of αSN in human CSF and bound to protein A/G agarose beads after co-IP using: anti-αSN antibody (sc-7011-R, Santa Cruz), the goat polyclonal anti-ApoE antibody (178479, EMD Millipore), mouse IgGs, rabbit IgGs, goat IgGs and the protein A/G agarose beads alone. (E) Detection of baseline level of αSN in human CSF and remained in the supernatant after co-IP using: anti-αSN antibody (sc-7011-R, Santa Cruz), the goat polyclonal anti-ApoE antibody (178479, EMD Millipore), mouse IgGs, rabbit IgGs, goat IgGs and the protein A/G agarose beads alone. 12 CSF samples were used for this experiment, both from Parkinson's disease cases (n=6, 3/3 males/females, average age 64.67±4.75) and healthy controls (n=6, 3/3 males/females, average age 66.50±1.38).

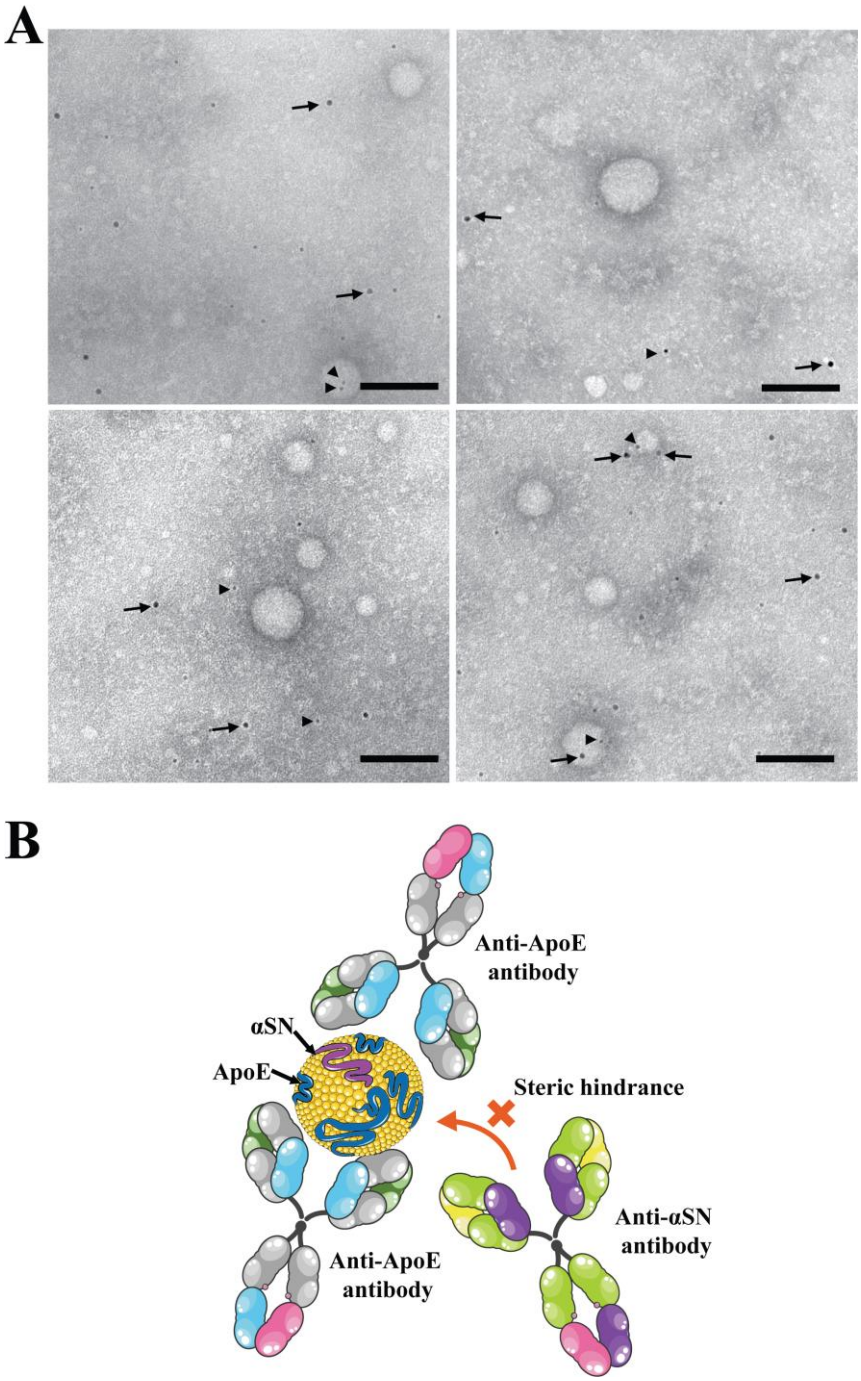


**Fig. S6. The TEM immuno-gold labelling analysis of the aSN and ApoE co-localization in the human CSF and a steric hindrance influencing the co-detection of αSN and ApoE.** (A) TEM images with the immuno-gold labelling against aSN (arrowhead) and ApoE (arrow) in the human CSF. Scale bars represent 100nm. (B) Image demonstrating a steric hindrance influencing the co-detection of αSN and ApoE on the same lipoprotein vesicles smaller than 20nm. Image prepared using Smart Servier Medical Art (https://smart.servier.com/), which is licensed under CC BY 3.0.

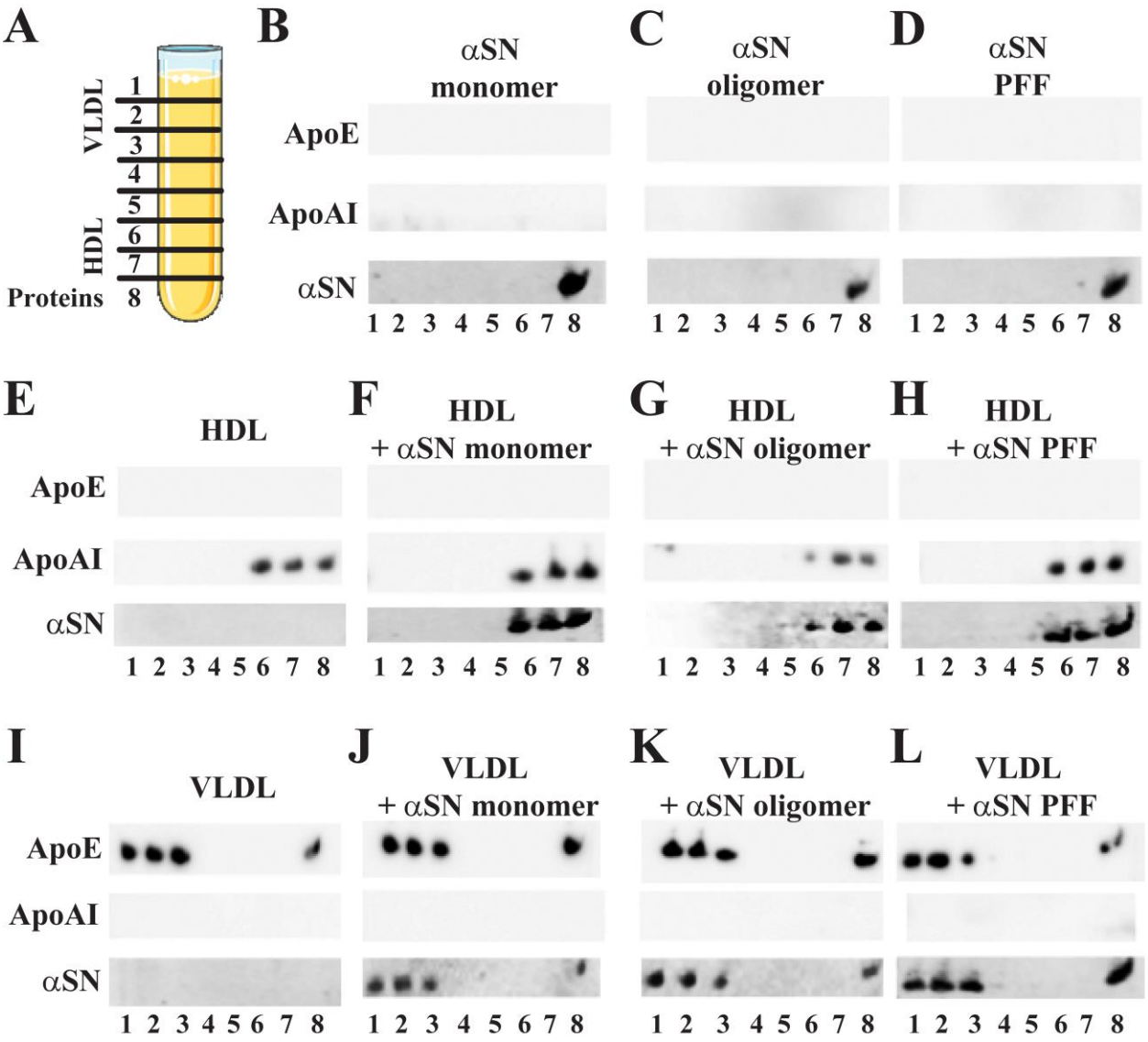


**Fig. S7. Interaction of αSN and lipoproteins analysed using separation of lipoproteins by density-gradient ultracentrifugation.** (A) Overview of density-gradient ultracentrifugation layers indicating VLDL, HDL and proteins distribution. (B-L) WB analysis of αSN, ApoE (VLDL) and ApoAI (HDL) in density-gradient ultracentrifugation separated fractions of: (B) monomeric αSN, (C) oligomeric αSN, (D) αSN PFF, (E) HDL, (F) HDL enriched with monomeric αSN, (G) HDL enriched with oligomeric αSN, (H) HDL enriched with PFF αSN, (I) VLDL, (J) VLDL enriched with monomeric αSN, (K) VLDL enriched with oligomeric αSN, (L) VLDL enriched with PFF αSN.

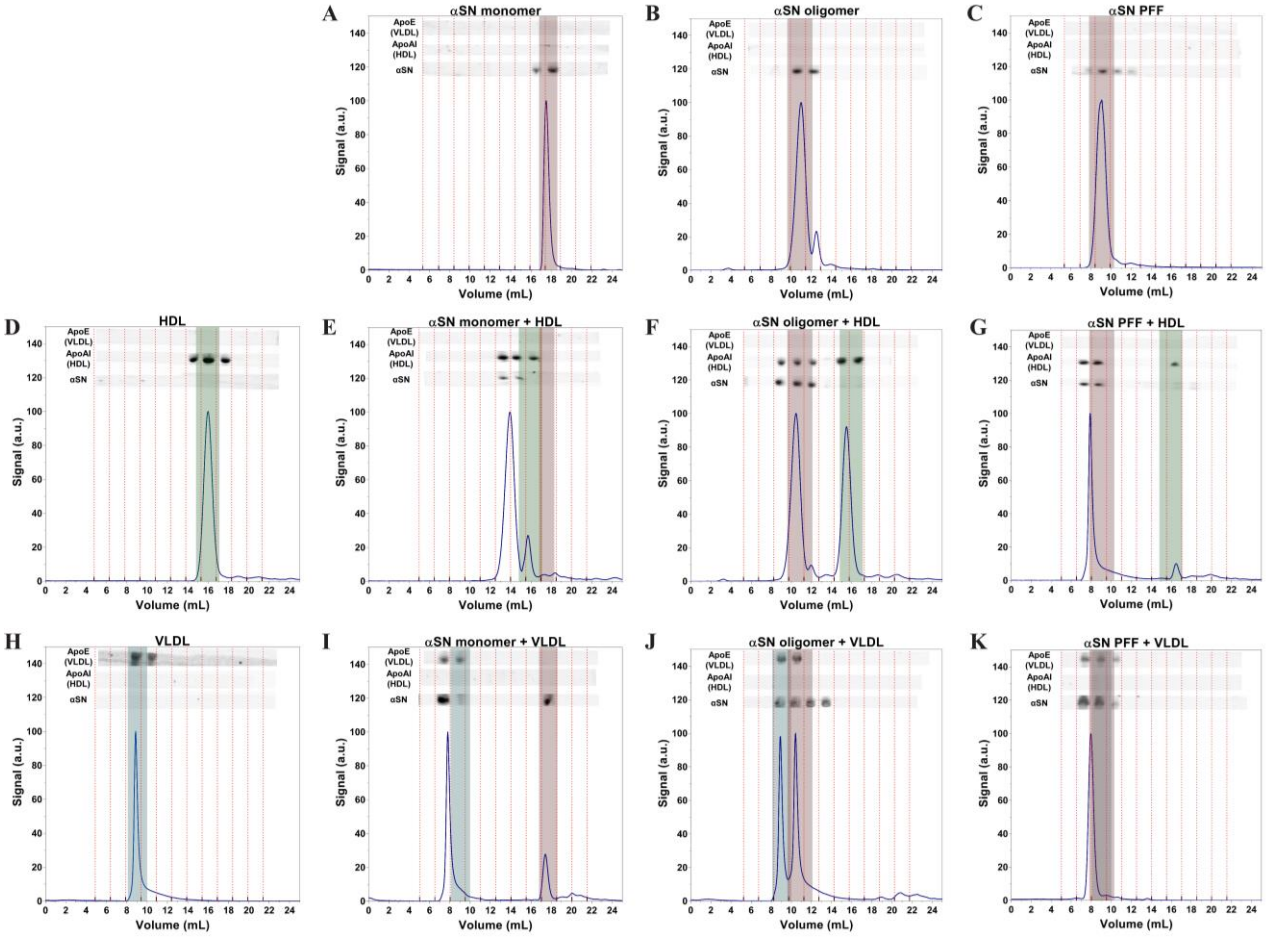


**Fig. S8. Interaction of αSN and lipoproteins analysed using size exclusion chromatography.** Elution profiles and WB analysis of: (A) monomeric αSN, (B) oligomeric αSN, (C) αSN PFF, (D) HDL, (E) HDL enriched with monomeric αSN, (F) HDL enriched with oligomeric αSN, (G) HDL enriched with PFF αSN, (H) VLDL, (I) VLDL enriched with monomeric αSN plasma, (J) VLDL enriched with oligomeric αSN, (K) VLDL enriched with PFF αSN. The red shaded panels represent elution volumes for non-interacting αSN monomers, oligomers and PFF, respectively. The green and blue shaded panels represent elution volumes for non-interacting HDL and VLDL particles, respectively. Inserts on top of each chromatogram represents WB analysis towards αSN, ApoE (VLDL) and ApoAI (HDL) in fractions collected during analysis.

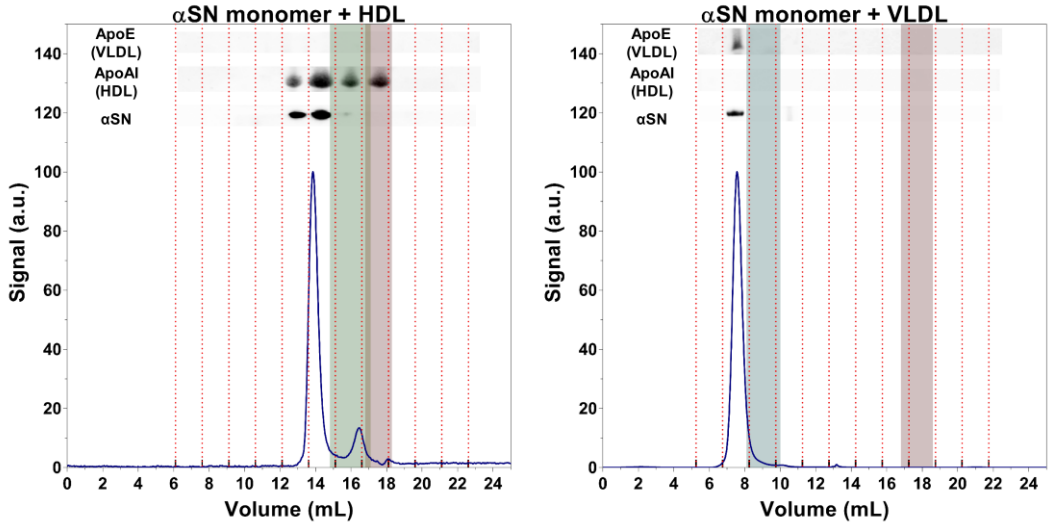


**Fig. S9. Analysis of αSN enriched lipoproteins using size exclusion chromatography and WB.** Elution profiles and WB analysis of presence of αSN, ApoE (VLDL) and ApoAI (HDL) in fractions obtained after isolation of αSN enriched lipoproteins by spin filetrs method. The red shaded panels represent elution volumes for non-interacting αSN monomers. The green and blue shaded panels represent elution volumes for non-interacting HDL and VLDL particles, respectively. Inserts on top of each chromatogram represents WB analysis towards αSN, ApoE (VLDL) and ApoAI (HDL) in fractions collected during analysis.

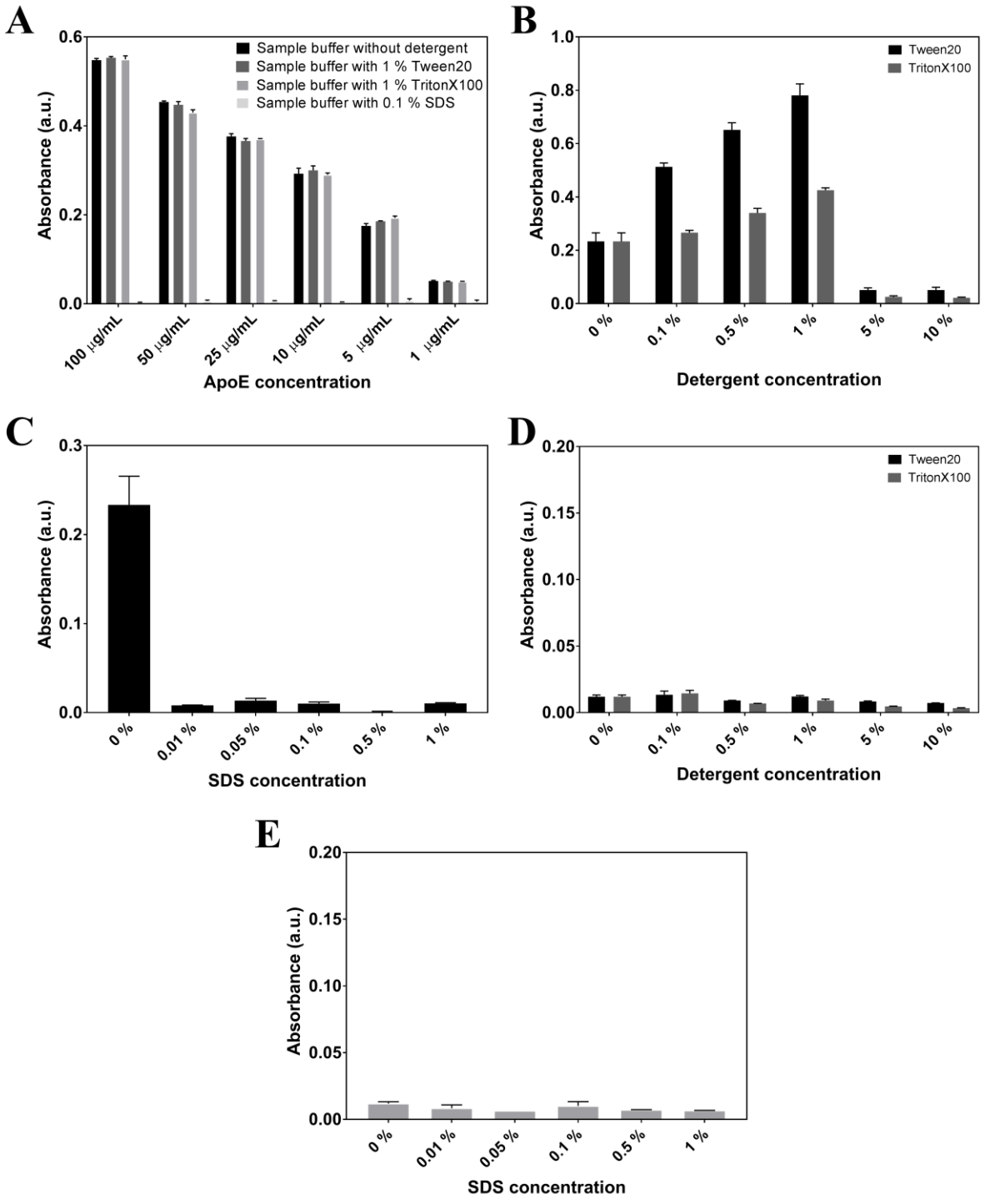


**Fig. S10. Performance of the ELISA method for detection of ApoE.** (A) Detection of the recombinant ApoE in a buffer containing different concentrations of most commonly used detergents (Tween-20, Triton X-100 and SDS). (B) Detection of CSF ApoE in buffer containing different concentrations of Tween-20, Triton X-100 and (C) SDS. (D) Background absorbance of buffer containing different concentrations of Tween-20, Triton X-100 and (E) SDS. The capture antibody – the goat polyclonal anti-ApoE antibody (178479, EMD Millipore). The detection antibody – the mouse monoclonal anti-ApoE antibody [E6D7] (ab1907, Abcam).

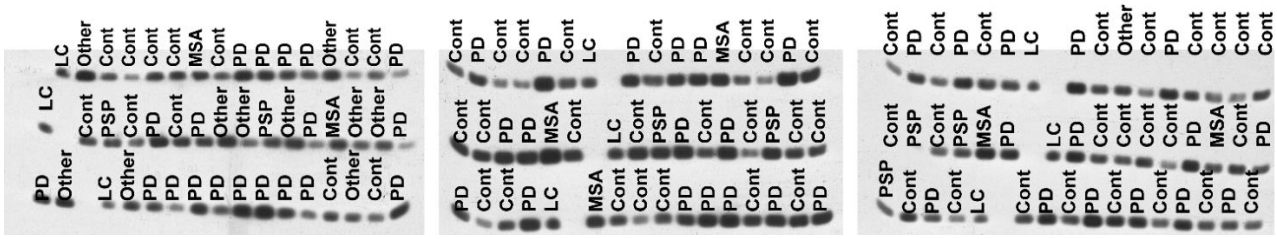


**Fig. S11. ApoE detection in the human CSF using the WB method.** Example representative autoradiograms obtained after running CSF samples and performing WB procedure against ApoE. LC – the loading control used to normalise signal across gels; PD – Parkinson's disease; MSA – multiple system atrophy; PSP – progressive supranuclear palsy; Cont – control.

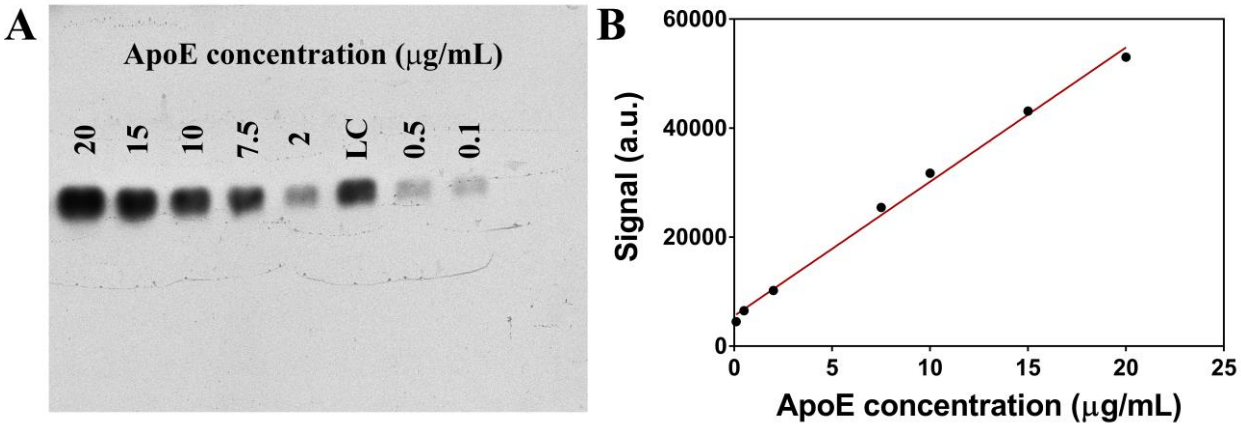


**Fig. S12. The recombinant ApoE standard curve used to estimate the absolute level of ApoE in Stockholm cohort.** (A) Autoradiograms obtained after performing WB procedure using several concentrations of the recombinant ApoE and the loading control (LC) used to normalise signal across gels in Stockholm cohort. (B) The standard curve obtained after analysing the signal obtained from autoradiogram of the recombinant ApoE ($R^2$=0.9954).

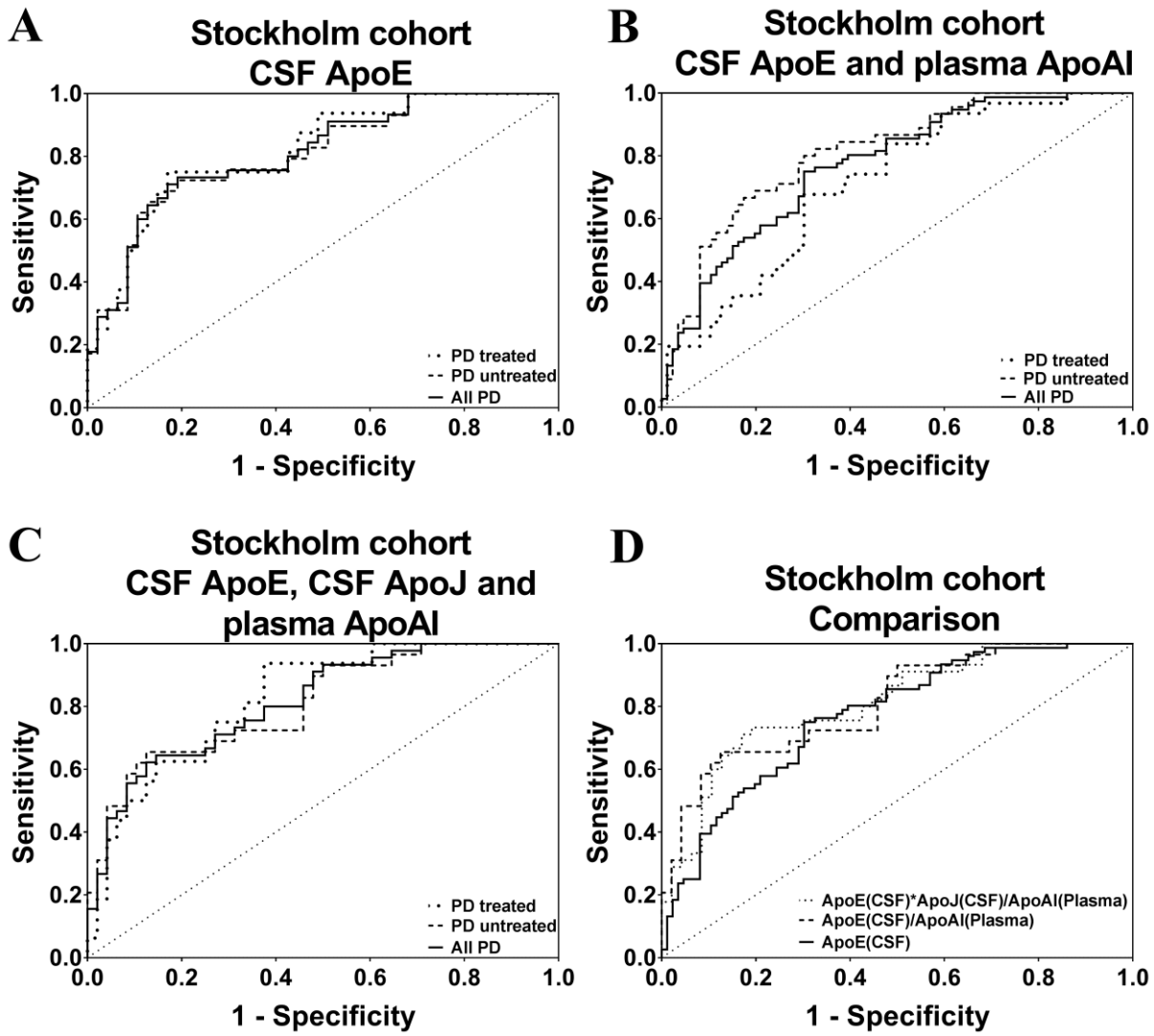


**Fig. S13. ROC curves showing the relationship between sensitivity (true positive) and 1 − specificity (true negative) in determining the predictive value of apolipoproteins' levels for Parkinson's disease.** (A) The ROC curve for the CSF ApoE level from the Stockholm cohort. A solid black line – the curve for all Parkinson's disease patients combined (the area under the ROC curve 0.77±0.04 with 95% confidence interval of 0.70–0.84); A dashed black line – the curve for untreated patients only (the area under the ROC curve 0.81±0.04 with 95% confidence interval of 0.74–0.88); and a dotted black line – curve for treated patients only (the area under the ROC curve 0.71±0.05 with 95% confidence interval of 0.61–0.81). (B) The ROC curve combining ApoE levels in CSF and ApoAI levels in plasma from the Stockholm cohort. A solid black line – the curve for all Parkinson's disease patients combined (the area under the ROC curve 0.81±0.04 with 95% confidence interval of 0.73–0.90); a dashed black line – the curve for untreated patients only (the area under the ROC curve 0.80±0.05 with 95% confidence interval of 0.71–0.90); and a dotted black line – curve for treated patients only (the area under the ROC curve 0.82±0.05 with 95% confidence interval of 0.72–0.93). (C) The ROC curve combining ApoE and ApoJ levels in CSF and ApoAI levels in plasma from the Stockholm cohort. A solid black line – the curve for all Parkinson's disease patients

combined (the area under the ROC curve 0.81±0.04 with 95% confidence interval of 0.72–0.90); A dashed black line – the curve for untreated patients only (the area under the ROC curve 0.80±0.05 with 95% confidence interval of 0.70–0.90); and a dotted black line – curve for treated patients only (the area under the ROC curve 0.81±0.06 with 95% confidence interval of 0.70–0.94). (D) The comparison of ROC curves from the Stockholm cohort: the CSF ApoE level (a solid line), ApoE and ApoAI (a dashed line) and ApoE, ApoJ and ApoAI (a dotted line) for all Parkinson's disease patients. PD: Parkinson's disease.

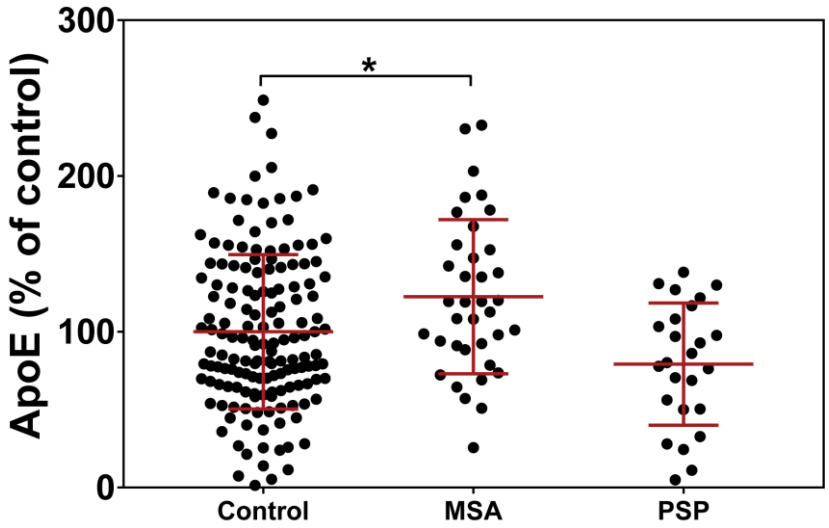


**Fig. S14. Levels of ApoE in CSF from MSA and PSP patients vs. healthy controls from combined Umeå and Lund Cohorts.** Tests used: the Kruskal-Wallis test with Dunn's correction for more than 2 groups. *p<0.05.

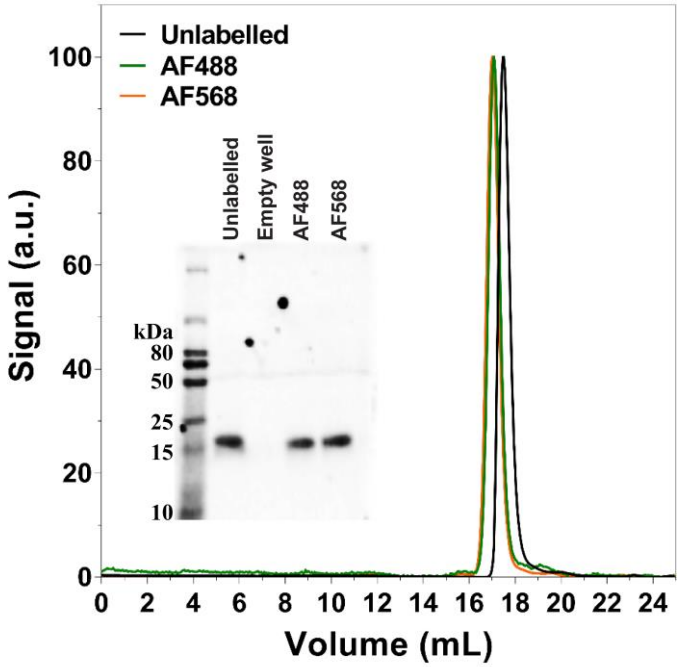


**Fig. S15. Analysis of stability of fluorescently labelled αSN using size exclusion chromatography and WB.** Elution profiles and WB analysis of fluorescently labelled and unlabelled αSN showing neither aggregation nor fragmentation of αSN after fluorescent labelling.

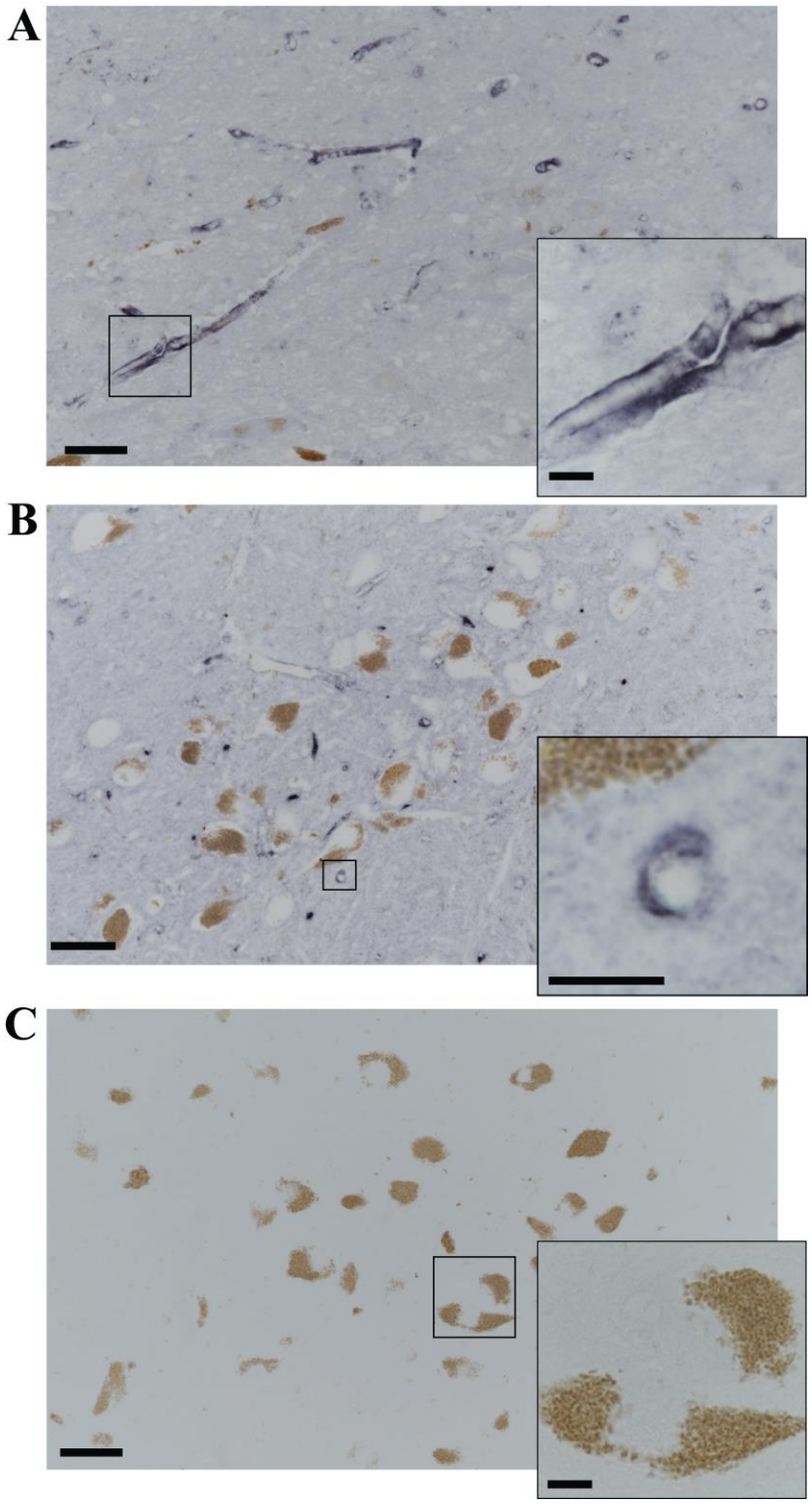


**Fig. S16. The negative control for the ApoE immunostaining and positive ApoE staining in proximity to veins in the human brain.** (A) The immunostaining against ApoE showing enriched localization of ApoE in a paravascular space in the human SN brain section. (B) The negative control for ApoE staining of human SN – omitting the primary antibody. Scale bars represent 50 μm (10 μm for inserts).

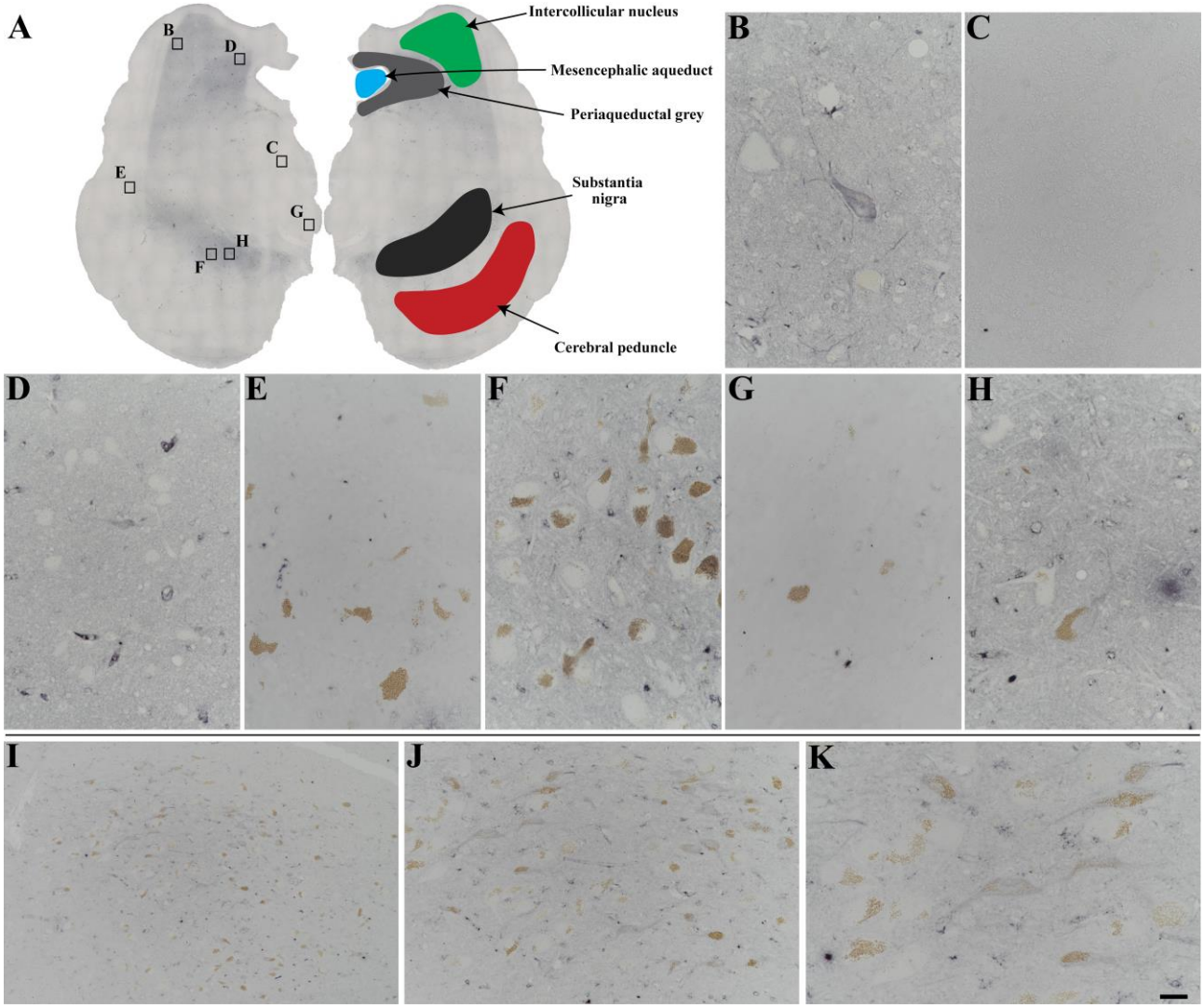


**Fig. S17. The distribution of ApoE immunoreactivity in control human brain stem.** (A) The representative human brain stem ApoE immunoreactivity overview of control individuals. (B-H) Rectangles denoted areas shown in higher magnification photomicrographs of intercollicular nucleus (B), tegmentum (C), periaqueductal grey (D), SN (E, F, H) and ventral tegmental area (G). The strongest ApoE signal, mainly located extracellularly, is observed in intercollicular nucleus, periaqueductal grey and SN region. (I-K) The close co-occurrence of both negative and positive ApoE dopaminergic cells detected in SN of control individuals. The brown, neuromelanin pigmented cells are dopaminergic neurons. The scale bar represents 25μm on B-H and K, 100μm on I and 50μm on J.

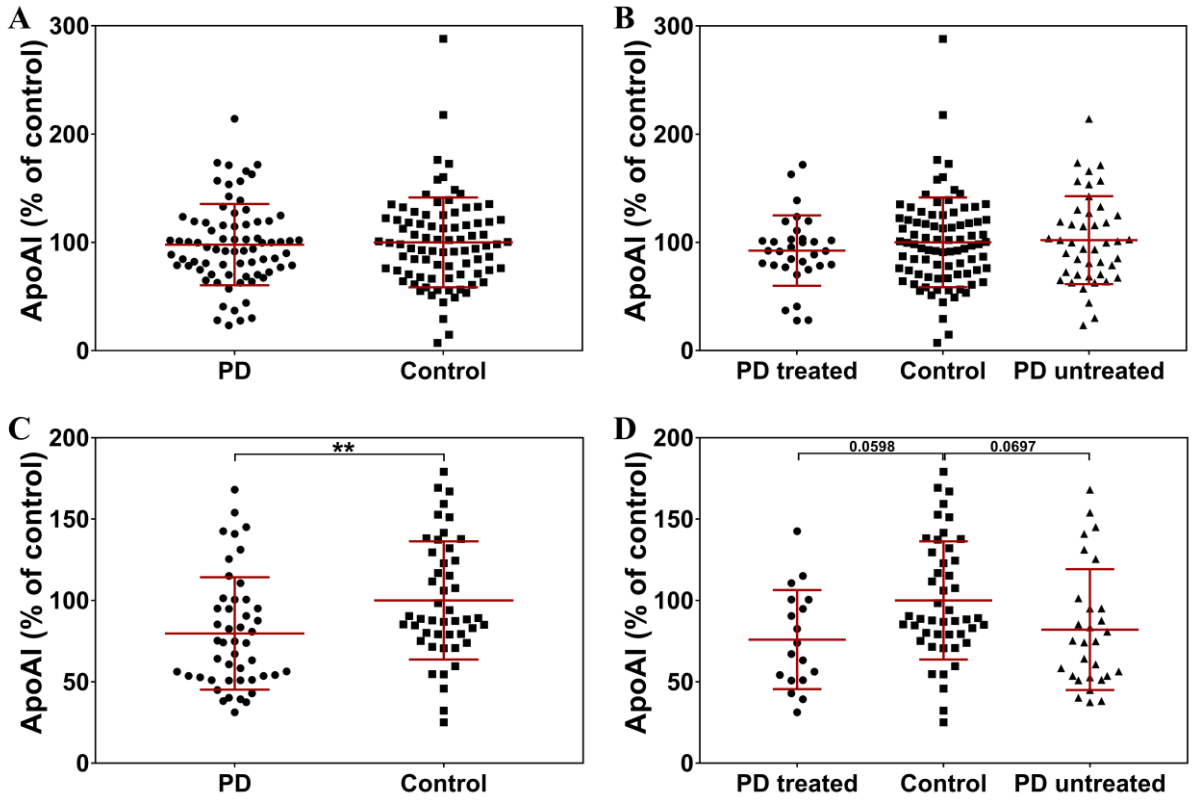


**Fig. S18. Levels of ApoAI in bio-fluids from the Stockholm Cohort.** (A) Levels of ApoAI in CSF of healthy controls compared to Parkinson's disease patients. (B) Levels of ApoAI in CSF of healthy controls compared to treated and untreated Parkinson's disease patients. (C) Levels of ApoAI in plasma of healthy controls compared to Parkinson's disease patients. (D) Levels of ApoAI in plasma of healthy controls compared to treated and untreated Parkinson's disease patients. Tests used: the Mann-Whitney test for 2 groups' comparison and the Kruskal-Wallis test with Dunn's correction for more than 2 groups. **p<0.01.

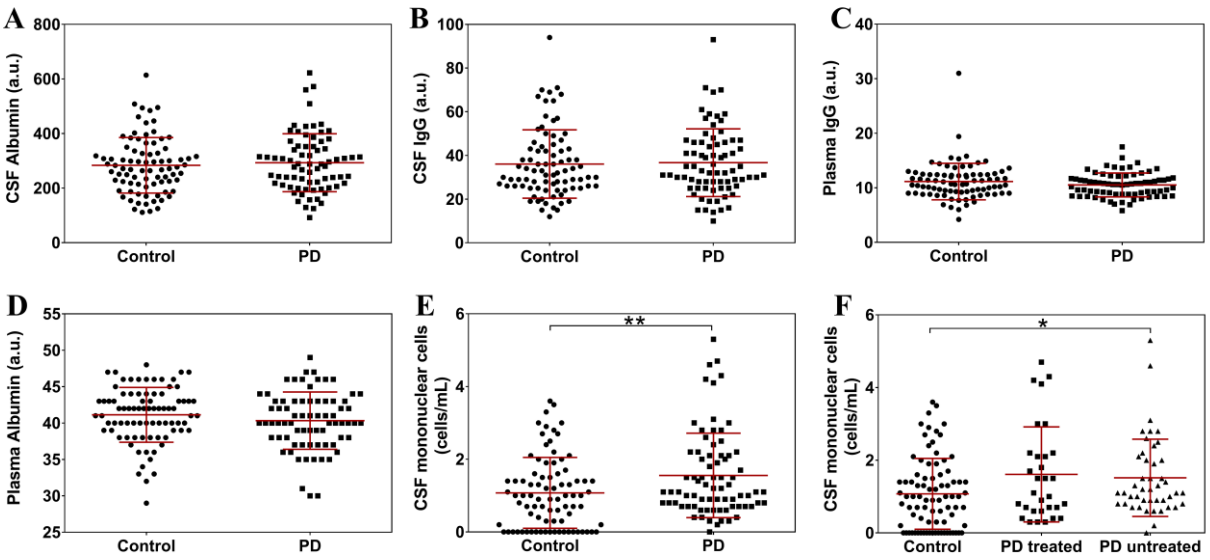


**Fig. S19. Comparison of Albumin, IgGs and mononuclear cells in CSF and plasma from the Stockholm cohort.** (A) Comparison of albumin levels in CSF from controls vs. Parkinson's disease patients. (B) Comparison of IgG levels in CSF from controls vs. Parkinson's disease patients. (C) Comparison of IgG levels in plasma from controls vs. Parkinson's disease patients. (D) Comparison of albumin levels in plasma from controls vs. Parkinson's disease patients. (E) Comparison of the mononuclear cells' count in CSF from controls vs. untreated and treated Parkinson's disease patients. (F) Comparison of the mononuclear cells count in CSF from controls vs. Parkinson's disease patients. Tests used: the Mann-Whitney test for 2 groups' comparison and the Kruskal-Wallis test with Dunn's correction for more than 2 groups. *$p<0.05$, **$p<0.01$.

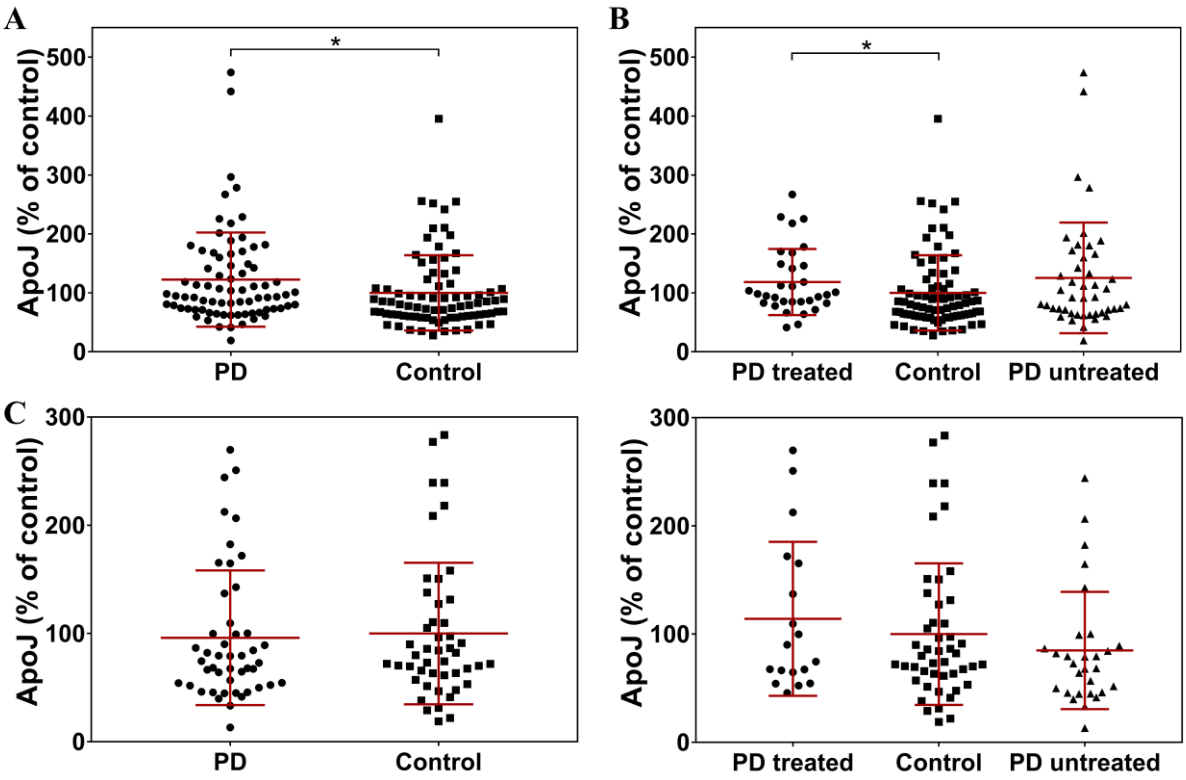


**Fig. S20**. **Levels of ApoJ in bio-fluids from the Stockholm Cohort.** (A) Levels of ApoJ in CSF of healthy controls compared to Parkinson's disease patients. (B) Levels of ApoJ in CSF of healthy controls compared to treated and untreated Parkinson's disease patients. (C) Levels of ApoJ in plasma of healthy controls compared to Parkinson's disease patients. (D) Levels of ApoJ in plasma of healthy controls compared to treated and untreated Parkinson's disease patients. Tests used: the Mann-Whitney test for 2 groups' comparison and the Kruskal-Wallis test with Dunn's correction for more than 2 groups. *$p<0.05$.

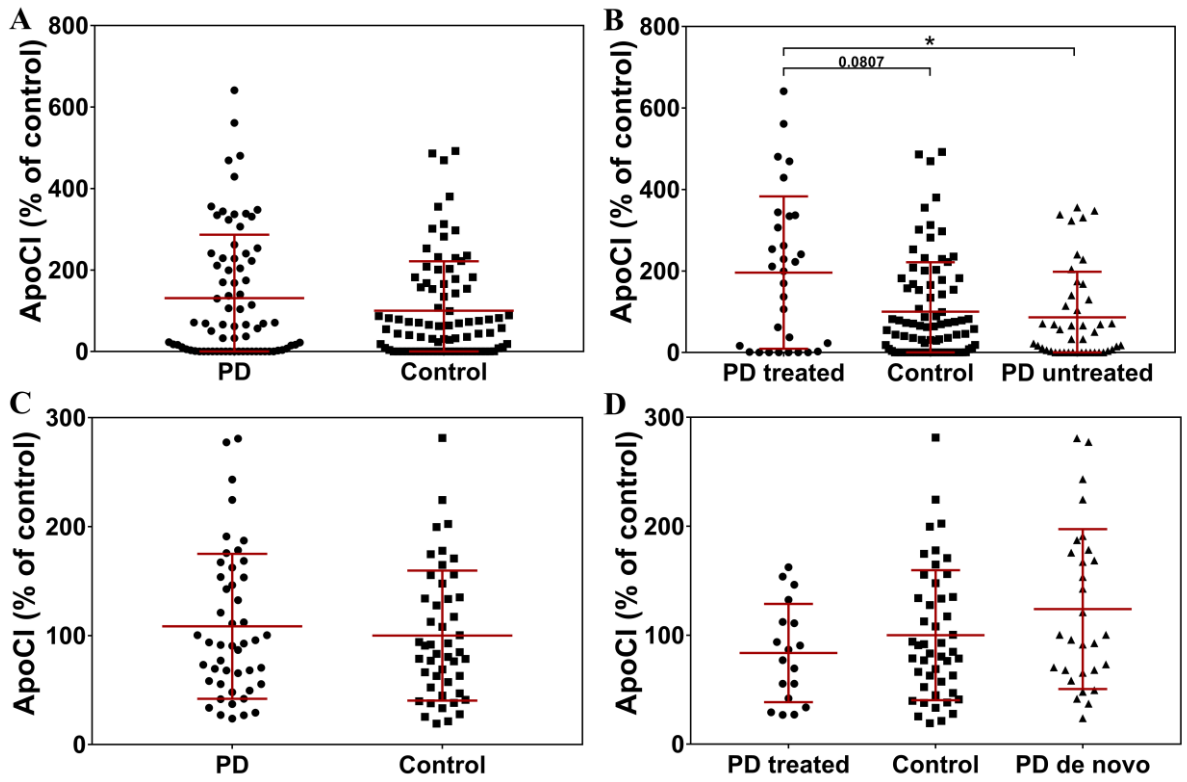


**Fig. S21. Levels of ApoCI in bio-fluids from the Stockholm cohort.** (A) Levels of ApoCI in CSF of healthy controls compared to Parkinson's disease patients. (B) Levels of ApoCI in CSF of healthy controls compared to treated and untreated Parkinson's disease patients. (C) Levels of ApoCI in plasma of healthy controls compared to Parkinson's disease patients. (D) Levels of ApoCI in plasma of healthy controls compared to treated and untreated Parkinson's disease patients. Tests used: the Mann-Whitney test for 2 groups' comparison and the Kruskal-Wallis test with Dunn's correction for more than 2 groups. *$p<0.05$.

**Table S1. Antibodies used in the study.** Details of antibodies used in presented study together with methods in which they were used.

| | Description | Conjugate | Host | Clonality | Cat. No. | Distributor | Method |
|---|---|---|---|---|---|---|---|
| **Primary** | anti-αSN | NA | rabbit | polyclonal | sc-7011-R | Santa Cruz | **co-IP, ELISA (capture), TEM** |
| | anti-αSN [LB 509] | NA | mouse | monoclonal | ab27766 | Abcam | **ELISA (detection), WB** |
| | anti-ApoE [E6D7] | NA | mouse | monoclonal | ab1907 | Abcam | **ELISA (detection), TEM, WB, IHC** |
| | anti-ApoE | NA | goat | polyclonal | 178479 | EMD Millipore | **co-IP, ELISA (capture)** |
| | anti-ApoAI [EP1368Y] | NA | rabbit | monoclonal | ab52945 | Abcam | **WB** |
| | anti-ApoCI | NA | mouse | monoclonal | ab54800 | Abcam | **WB** |
| | anti-ApoJ [EPR2911] | NA | rabbit | monoclonal | ab92548 | Abcam | **WB** |
| | goat IgGs | NA | goat | polyclonal | I5256 | Sigma | **co-IP, ELISA (capture)** |
| | mouse IgGs | NA | mouse | polyclonal | I5381 | Sigma | **co-IP** |
| | rabbit IgGs | NA | rabbit | polyclonal | I5006 | Sigma | **co-IP, ELISA (capture)** |
| **Secondary** | anti-mouse IgGs | HRP | goat | polyclonal | P0447 | Dako | **WB, ELISA, IHC** |
| | anti-rabbit IgGs | HRP | goat | polyclonal | P0448 | Dako | **WB** |

**Table S2. Basic demographics of studied subjects.** Age (mean ±standard deviation) and gender of Parkinson's disease (PD), MSA, PSP patients and matched controls. In the Stockholm Cohort both CSF and plasma were analysed. For the Stockholm cohort, for each plasma sample, the CSF sample from the same patient was analysed enabling a correlation analysis. For Umeå and Lund cohorts only CSF was analysed. Both treated and untreated patients were included in the study.

| | Stockholm Cohort CSF | | | | Stockholm Cohort Plasma | | | |
|---|---|---|---|---|---|---|---|---|
| | **PD** | **Control** | **PD treated** | **PD untreated** | **PD** | **Control** | **PD treated** | **PD untreated** |
| **Age (Mean ±SD)** | 63,23 ±10,80 | 63,29 ±9,31 | 64,78 ±8,24 | 62,13 ±12,18 | 65,83 ±6,52 | 63,68 ±10,38 | 61,52 ±14,01 | 63,17 ±11,91 |
| **Number of subjects** | 77 | 86 | 32 | 45 | 47 | 47 | 18 | 29 |
| **Males** | 54 | 60 | 22 | 32 | 36 | 36 | 14 | 22 |
| **Females** | 23 | 26 | 10 | 13 | 11 | 11 | 4 | 7 |

| | Umeå Cohort CSF only | | | | Lund Cohort CSF only | | | |
|---|---|---|---|---|---|---|---|---|
| | **PD** | **Control** | **MSA** | **PSP** | **PD** | **Control** | **MSA** | **PSP** |
| **Age (Mean ±SD)** | 70,67 ±9,50 | 68,40 ±8,08 | 72,54 ±7,54 | 75,38 ±2,55 | 66,00 ±7,45 | 66,26 ±7,32 | 63,32 ±8,27 | 70,59 ±6,33 |
| **Number of subjects** | 64 | 102 | 11 | 8 | 46 | 46 | 27 | 17 |
| **Males** | 40 | 57 | 7 | 3 | 22 | 22 | 13 | 8 |
| **Females** | 24 | 45 | 4 | 5 | 24 | 24 | 14 | 9 |

| | Umeå Cohort CSF only | | Lund Cohort CSF only | |
|---|---|---|---|---|
| | **PD treated** | **PD untreated** | **PD treated** | **PD untreated** |
| **Age (Mean ±SD)** | 70,99 ±9,82 | 70,65 ±8,94 | 64,13 ±7,88 | 68,39 ±6,00 |
| **Number of subjects** | 26 | 38 | 23 | 23 |
| **Males** | 16 | 24 | 11 | 11 |
| **Females** | 10 | 14 | 12 | 12 |

**Table S3. Average Parkinson's disease severity scores of studied subjects.** Average severity scores (mean ±standard deviation) for Parkinson's disease (PD) patients. In the Stockholm Cohort both the CSF and plasma were analysed. Both treated and untreated patients were included in the study. For this cohort, for each plasma sample, a CSF sample from the same patient was analysed enabling correlation analysis. For Umeå and Lund cohorts only CSF was analysed. Both treated and untreated patients were included.

| | | PD | PD treated | PD untreated |
|---|---|---|---|---|
| **Stockholm cohort (CSF)** | **LED** | 273.28±403.84 | 664.98±368.04 | 0.00±0.00 |
| | **MoCA** | 23.88±4.21 | 22.54±4.40 | 24.85±3.85 |
| | **Hoehn&Yahr** | 1.83±0.72 | 2.08±0.62 | 1.65±0.74 |
| | **NMS** | 7.08±4.04 | 8.83±3.56 | 6.32±4.06 |
| | **UPDRS Total** | 36.19±16.03 | 43.40±17.85 | 33.94±15.02 |
| | **BDI** | 9.84±6.86 | 7.77±4.99 | 10.92±7.52 |
| | **Disease duration** | 2.84±4.80 | 6.84±5.32 | 0.00±0.00 |
| | **Age at onset** | 63.23±10.87 | 64.78±8.37 | 62.13±12.32 |
| **Stockholm cohort (Plasma)** | **LED** | 290.32±429.56 | 758.06±351.95 | 0.00±0.00 |
| | **MoCA** | 23.69±4.07 | 22.00±4.15 | 24.64±0.76 |
| | **Hoehn&Yahr** | 1.91±0.75 | 2.19±0.62 | 1.74±0.14 |
| | **NMS** | 6.44±4.37 | 9.00±6.24 | 6.09±0.88 |
| | **UPDRS Total** | 35.78±16.30 | 57.00±19.00 | 33.13±2.91 |
| | **BDI** | 10.43±7.40 | 7.00±4.58 | 10.95±1.72 |
| | **Disease duration** | 3.00±4.81 | 7.83±4.76 | 0.00±0.00 |
| | **Age at onset** | 63.40±12.22 | 66.44±7.29 | 61.52±14.26 |
| **Umeå cohort** | **LED** | 143.71±205.34 | 353.75±170.23 | 0.00±0.00 |
| | **UPDRS3** | 26.86±10.66 | 24.23±10.18 | 28.66±10.74 |
| | **Hoehn&Yahr** | 2.30±0.47 | 2.23±0.41 | 2.36±0.51 |
| | **Schwab& England** | 87.53±7.36 | 88.20±6.75 | 87.08±7.80 |
| | **Disease Duration** | 2.55±1.56 | 3.61±1.70 | 1.82±0.92 |
| | **Age at onset** | 70.84±9.53 | 70.99±9.12 | 70.65±9.95 |
| **Lund cohort** | **LED** | 351.51±440.96 | 703.01±373.33 | 0.00±0.00 |
| | **UPDRS3** | 17.70±11.26 | 17.48±10.35 | 17.91±12.34 |
| | **Hoehn&Yahr** | 1.84±0.83 | 1.75±0.68 | 1.90±0.94 |
| | **Schwab& England** | 89.78±9.37 | 90.00±7.98 | 89.57±10.76 |
| | **Disease Duration** | 4.57±4.42 | 7.04±4.66 | 1.86±1.82 |

**Table S4. Correlation of apolipoproteins levels to Parkinson's disease treatment, severity scores and disease duration in both CSF and plasma samples from the Stockholm Cohort**. p-values <0.05 and corresponding r-values are marked with bold font. LEDD, Levodopa Equivalent Daily Doses; MoCA - Montreal Cognitive Assessment; BDI - Beck's Depression Inventory; NMSQ - Non-motor symptoms questionnaire.

| | | | LEDD | Hoehn& Yahr | UPDRS part 3 | Age at onset | Disease duration | MoCA | NMSQ | BDI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stockholm Cohort | | | | | | | |
| CSF | ApoAI | p | 0.5554 | 0.2747 | 0.3753 | 0.2600 | 0.9307 | 0.8878 | 0.6496 | 0.4012 |
| | | r | 0.1082 | -0.1269 | 0.1386 | 0.1308 | 0.0160 | 0.0186 | 0.0731 | 0.1383 |
| | ApoCI | p | 0.1687 | 0.2133 | 0.4065 | 0.3669 | **0.0299** | 0.2974 | 0.9838 | 0.1823 |
| | | r | 0.2626 | 0.1464 | 0.1315 | 0.1049 | **0.3904** | -0.1392 | -0.0033 | -0.2210 |
| | ApoE | p | **0.0390** | 0.3682 | 0.2250 | 0.9137 | 0.3434 | 0.4023 | 0.1626 | 0.1717 |
| | | r | **-0.2422** | -0.1061 | -0.1912 | -0.0126 | -0.1761 | 0.1131 | -0.2251 | -0.2264 |
| | ApoJ | p | 0.5703 | 0.1706 | 0.1832 | 0.6572 | 0.5693 | 0.6372 | 0.0577 | 0.8330 |
| | | r | 0.1042 | -0.1578 | -0.2069 | -0.0514 | 0.1045 | 0.0621 | -0.2988 | 0.0349 |
| Plasma | ApoAI | p | 0.2940 | 0.7963 | 0.9024 | 0.1307 | 0.4360 | 0.7090 | 0.9226 | 0.3726 |
| | | r | -0.2618 | 0.0387 | 0.0248 | 0.3812 | -0.1959 | 0.0617 | -0.0205 | 0.1950 |
| | ApoCI | p | 0.0878 | 0.7553 | 0.1308 | 0.7438 | **0.0057** | 0.1463 | 0.8602 | **0.0018** |
| | | r | 0.4139 | 0.0467 | -0.2982 | 0.0490 | **0.6238** | 0.2370 | 0.0371 | **0.6137** |
| | ApoE | p | 0.7194 | 0.8030 | 0.7058 | 0.4849 | 0.1262 | 0.6219 | 0.9883 | 0.1143 |
| | | r | 0.0910 | -0.0374 | 0.0762 | -0.1044 | 0.3741 | -0.0815 | 0.0031 | 0.3384 |
| | ApoJ | p | 0.1697 | 0.1876 | 0.2241 | **0.0380** | 0.1594 | 0.5814 | 0.7987 | 0.4471 |
| | | r | -0.3383 | -0.1956 | -0.2419 | **-0.3036** | -0.3461 | 0.0910 | -0.0537 | -0.1667 |

**Table S5. Correlations of apolipoproteins levels to age and biochemical components**. Both CSF and plasma samples from Stockholm Cohort Parkinson's disease patients and matched controls were analysed. p-values <0.05 and corresponding r-values are marked with a bold font.

| | | | | Age | CSF erythro-cytes | CSF leukocytes | CSF polynu-clear cells | CSF mono-nuclear cells | Albumin ratio | CSF albumin | Plasma albumin | IgG index | CSF IgG | Plasma IgG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CSF** | **Control** | **ApoAI** | p | 0.3723 | 0.9496 | 0.9071 | 0.5441 | 0.9084 | **< 0.0001** | **< 0.0001** | -------- | 0.4061 | **0.0004** | -------- |
| | | | r | -0.0974 | -0.0070 | 0.0129 | 0.0667 | 0.0127 | **0.5209** | **0.5783** | -------- | 0.0913 | **0.3754** | -------- |
| | | **ApoCI** | p | 0.7206 | 0.1950 | **0.0062** | 0.5675 | **0.0061** | **< 0.0001** | **< 0.0001** | -------- | 0.1363 | **0.0052** | -------- |
| | | | r | 0.0391 | 0.1419 | **0.2946** | 0.0629 | **0.2949** | **0.5184** | **0.5141** | -------- | 0.1649 | **0.3042** | -------- |
| | | **ApoE** | p | 0.1477 | 0.3224 | 0.1692 | 0.5714 | 0.1691 | 0.5237 | 0.8850 | -------- | 0.3530 | 0.7535 | -------- |
| | | | r | -0.1574 | -0.1086 | 0.1505 | -0.0623 | 0.1505 | 0.0701 | 0.0159 | -------- | -0.1020 | -0.0346 | -------- |
| | | **ApoJ** | p | 0.9152 | 0.4111 | 0.6589 | 0.3284 | 0.6543 | 0.8068 | 0.7452 | -------- | 0.0630 | 0.7016 | -------- |
| | | | r | 0.0119 | 0.0926 | -0.0498 | 0.1100 | -0.0505 | 0.0276 | 0.0367 | -------- | 0.2076 | 0.0432 | -------- |
| | **PD** | **ApoAI** | p | 0.2600 | 0.9873 | 0.3076 | 0.7012 | 0.2367 | **< 0.0001** | **< 0.0001** | -------- | 0.6249 | **< 0.0001** | -------- |
| | | | r | 0.1308 | -0.0019 | 0.1194 | 0.0450 | 0.1383 | **0.6386** | **0.6184** | -------- | 0.0578 | **0.5674** | -------- |
| | | **ApoCI** | p | 0.4164 | 0.2208 | 0.1649 | 0.2120 | 0.1819 | **< 0.0001** | **0.0002** | -------- | 0.4304 | **0.0184** | -------- |
| | | | r | 0.0952 | 0.1431 | 0.1620 | 0.1458 | 0.1558 | **0.4556** | **0.4291** | -------- | 0.0937 | **0.2753** | -------- |
| | | **ApoE** | p | 0.9137 | 0.3765 | 0.8251 | 0.4768 | 0.8564 | 0.1571 | 0.2333 | -------- | 0.4265 | 0.1365 | -------- |
| | | | r | -0.0126 | 0.1036 | 0.0260 | -0.0834 | 0.0212 | 0.1673 | 0.1412 | -------- | -0.0945 | 0.1759 | -------- |
| | | **ApoJ** | p | 0.6572 | 0.5065 | 0.3894 | 0.7862 | 0.3502 | 0.4669 | 0.4076 | -------- | 0.1863 | 0.3897 | -------- |
| | | | r | -0.0514 | -0.0774 | 0.1001 | -0.0316 | 0.1086 | -0.0853 | -0.0970 | -------- | -0.1543 | -0.1008 | -------- |
| **Plasma** | **Control** | **ApoAI** | p | 0.3894 | -------- | -------- | -------- | -------- | 0.6130 | -------- | 0.3266 | 0.7779 | -------- | 0.0685 |
| | | | r | -0.1285 | -------- | -------- | -------- | -------- | 0.0757 | -------- | 0.1463 | -0.0423 | -------- | -0.2681 |
| | | **ApoCI** | p | 0.9059 | -------- | -------- | -------- | -------- | 0.3669 | -------- | **0.0465** | 0.8499 | -------- | **0.0177** |
| | | | r | 0.0177 | -------- | -------- | -------- | -------- | -0.1346 | -------- | **0.2919** | -0.0284 | -------- | **-0.3445** |
| | | **ApoE** | p | 0.8925 | -------- | -------- | -------- | -------- | 0.7454 | -------- | 0.3588 | 0.4928 | -------- | 0.3487 |
| | | | r | 0.8925 | -------- | -------- | -------- | -------- | -0.0486 | -------- | -0.1369 | 0.1025 | -------- | -0.1398 |
| | | **ApoJ** | p | 0.6333 | -------- | -------- | -------- | -------- | 0.3823 | -------- | 0.2753 | 0.4596 | -------- | 0.1706 |
| | | | r | -0.0714 | -------- | -------- | -------- | -------- | -0.1304 | -------- | 0.1624 | -0.1105 | -------- | -0.2032 |
| | **PD** | **ApoAI** | p | 0.4556 | -------- | -------- | -------- | -------- | 0.6130 | -------- | 0.1883 | 0.1370 | -------- | 0.9672 |
| | | | r | 0.1115 | -------- | -------- | -------- | -------- | -0.0766 | -------- | 0.1975 | -0.2226 | -------- | -0.0062 |
| | | **ApoCI** | p | 0.7341 | -------- | -------- | -------- | -------- | 0.8070 | -------- | **0.0027** | **0.0063** | -------- | 0.7703 |
| | | | r | 0.0509 | -------- | -------- | -------- | -------- | -0.0370 | -------- | **0.4325** | **-0.3969** | -------- | 0.0442 |
| | | **ApoE** | p | 0.6622 | -------- | -------- | -------- | -------- | 0.9981 | -------- | **0.0263** | 0.9353 | -------- | 0.6350 |
| | | | r | 0.6622 | -------- | -------- | -------- | -------- | -0.0004 | -------- | **0.3276** | -0.0123 | -------- | 0.0719 |
| | | **ApoJ** | p | **0.0165** | -------- | -------- | -------- | -------- | 0.0837 | -------- | **0.0062** | **0.0138** | -------- | 0.2670 |
| | | | r | **-0.3482** | -------- | -------- | -------- | -------- | -0.2578 | -------- | **-0.3976** | **0.3608** | -------- | -0.1671 |

**Table S6. Correlation of apolipoproteins levels in both CSF and plasma samples from Stockholm Cohort Parkinson's disease patients and matched controls.** p-values (a normal font – the upper right half of the table) <0.05 and corresponding r-values (an underlined font – the bottom left half of the table) are marked with bold font.

| | | | **p-values** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Plasma** | | | | **CSF** | | | |
| | | | **ApoAI** | **ApoCI** | **ApoE** | **ApoJ** | **ApoAI** | **ApoCI** | **ApoE** | **ApoJ** |
| **r-values** | **Plasma** | **ApoAI** | | 0.1182 | 0.1645 | **0.0485** | 0.4149 | 0.4804 | **0.0468** | 0.4138 |
| | | **ApoCI** | 0.1622 | | **0.0017** | 0.5424 | 0.2848 | 0.1263 | 0.9754 | 0.3917 |
| | | **ApoE** | -0.1445 | **0.3202** | | 0.3732 | 0.4682 | 0.9378 | 0.9958 | 0.2733 |
| | | **ApoJ** | **0.2041** | -0.0636 | -0.0929 | | 0.3843 | 0.8818 | 0.3328 | 0.6508 |
| | **CSF** | **ApoAI** | 0.0855 | 0.1121 | -0.0761 | -0.0913 | | **<0.0001** | 0.7475 | 0.5203 |
| | | **ApoCI** | -0.0741 | 0.1597 | 0.0082 | -0.0156 | **0.5024** | | 0.9300 | 0.7512 |
| | | **ApoE** | **-0.2067** | -0.0032 | -0.0006 | 0.1015 | -0.0338 | 0.0092 | | 0.7784 |
| | | **ApoJ** | -0.0867 | 0.0909 | 0.1161 | -0.0481 | -0.0683 | -0.0337 | -0.0299 | |

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