SUPPLEMENTARY MATERIAL

Addressing inter individual variability in CSF levels of brain derived proteins across neurodegenerative diseases

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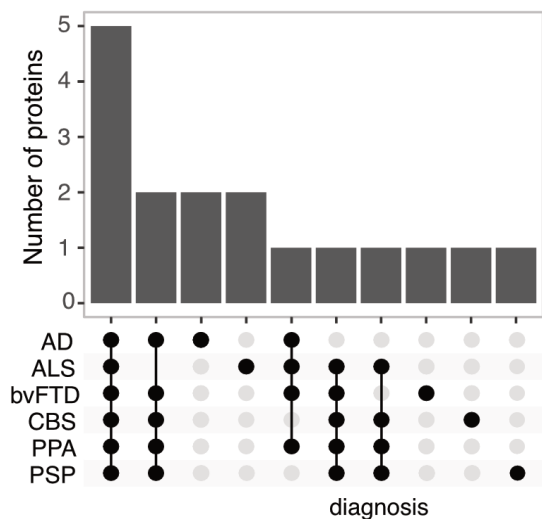
Supplementary figures and tables

Supplementary figures are listed first, followed by supplementary tables.
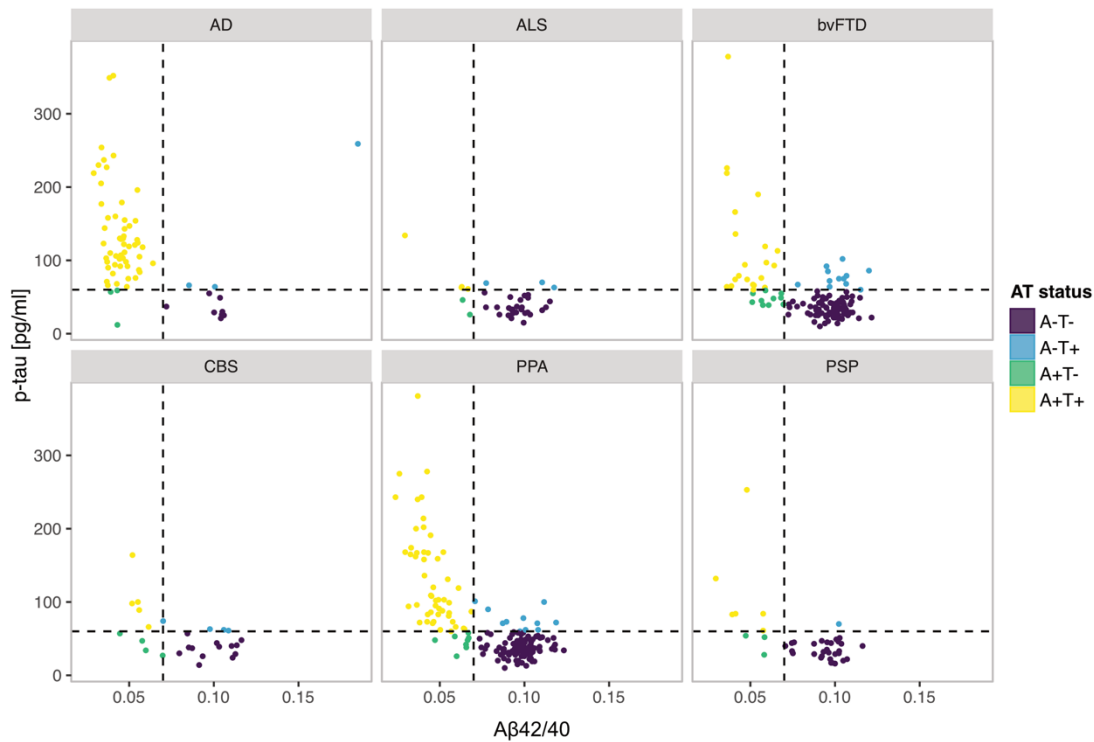
Supplementary figure 1: CSF Aβ42/40 and p-tau levels per diagnosis. The dashed lines label the classification cut-offs: Aβ42/40 = 0.07; p-tau = 60 pg/ml.
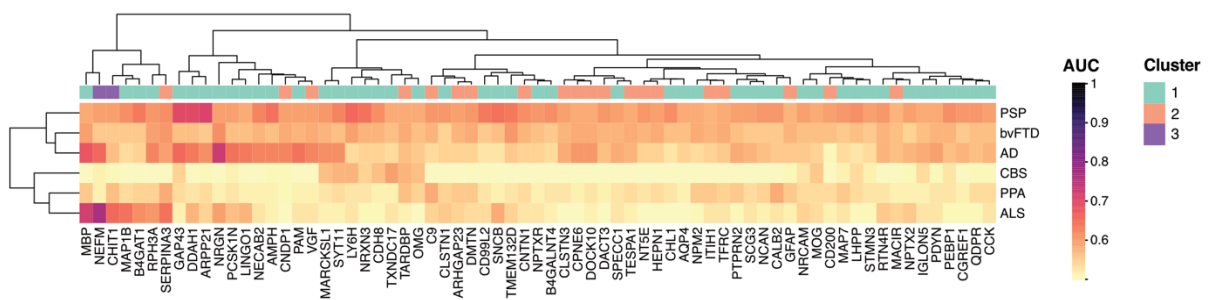
Supplementary figure 2: Disease specificity of CSF proteins. A heatmap showing predictive accuracy (evaluated with AUC) of individual proteins for each disease vs. all diseases, with adjustment for median CSF protein levels. The predicted disease is indicated on the right. The heatmap is annotated on top with the clustering results based on protein correlation (Fig. 1A).

Supplementary figure 3: Top 10 proteins with best performance in classifying disease vs. healthy controls – overlap between diseases
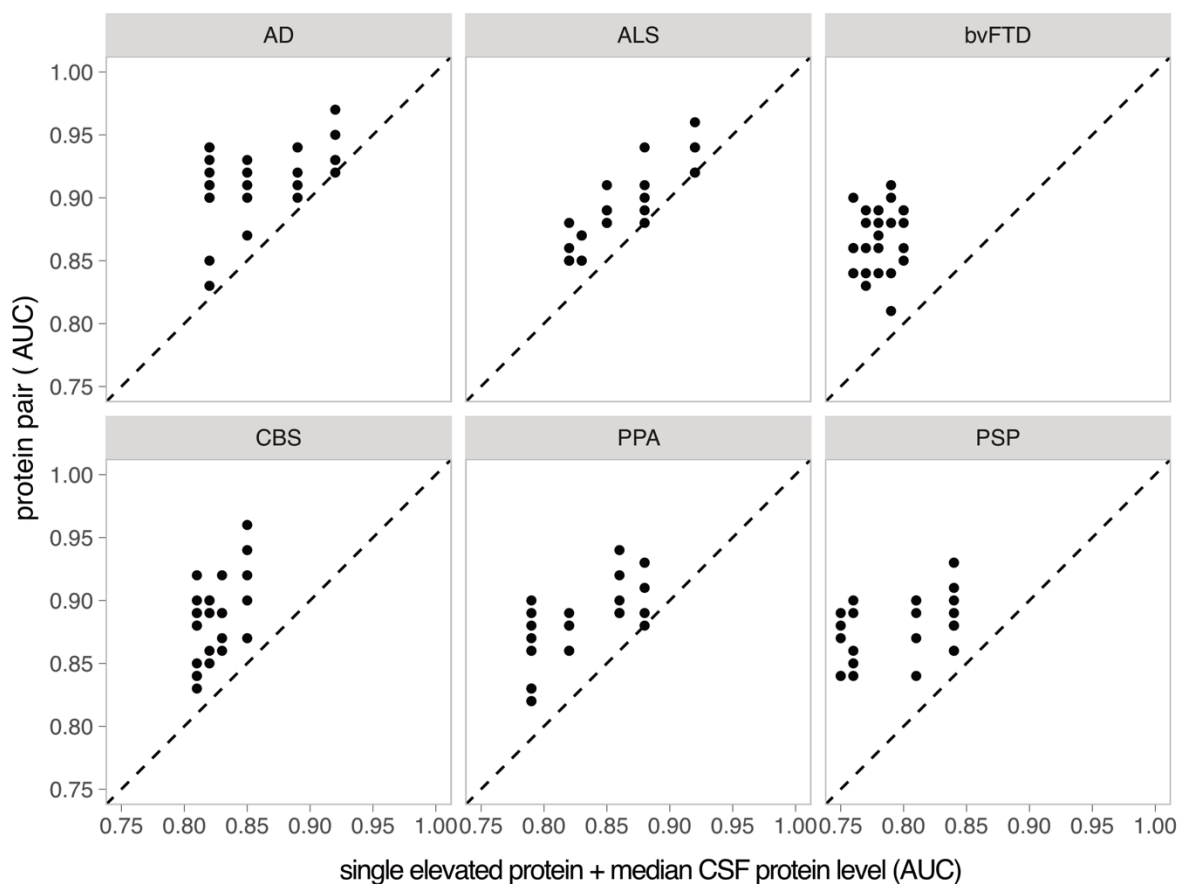
Supplementary figure 4: AUC obtained from logistic regression models for disease versus healthy controls classification. The x-axis represents the AUC values obtained from models with single proteins *elevated* in disease and CSF median protein levels as predictors. On the y-axis, AUC values are depicted for models with protein pairs, where one protein is elevated and one is decreased in disease, as predictors.
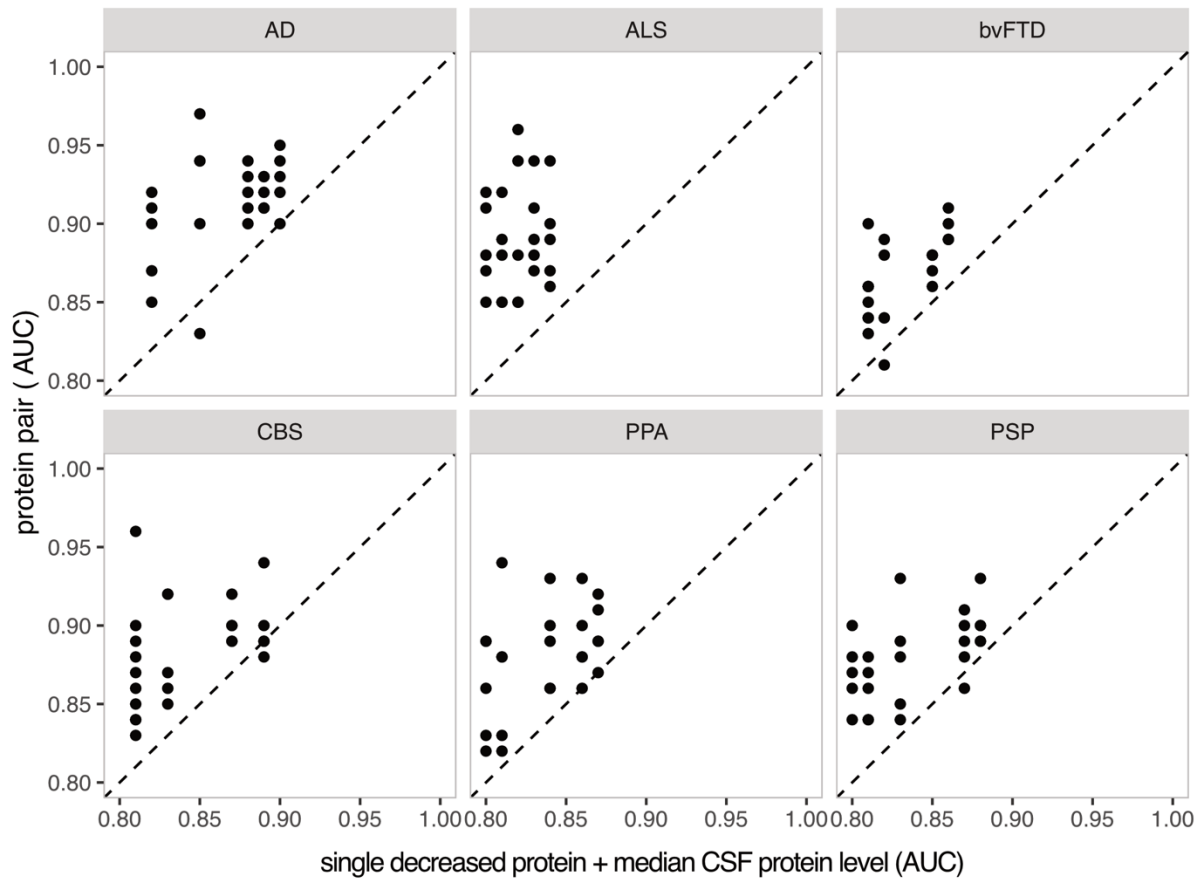
Supplementary figure 5: AUC obtained from logistic regression models for disease versus healthy controls classification. The x-axis represents the AUC values obtained from models with single proteins *decreased* in disease and CSF median protein levels as predictors. On the y-axis, AUC values are depicted for models with protein pairs, where one protein is elevated and one is decreased in disease, as predictors.

Supplementary table 1: List of analysed proteins and used antibodies.

HGNC ID	Protein name	Uniprot ID	Antibody	Sample dilution
AMPH	Amphiphysin	P49418	HPA019829	1:25
AQP4	Aquaporin 4	P55087	HPA014784	1:200
ARHGAP23	Rho GTPase activating protein 23	Q9P227	HPA019818	1:25
ARPP21	CAMP regulated phosphoprotein 21	Q9UBL0	HPA017303	1:25
B4GALNT4	Beta-1,4-N-acetyl-galactosaminyltransferase 4	Q76KP1	HPA053126	1:25
B4GAT1	Beta-1,4-glucuronyltransferase 1	O43505	HPA015484	1:200
C9	Complement C9	P02748	HPA070709	1:25
CALB2	Calbindin 2	P22676	HPA007305	1:25
CCK	Cholecystokinin	P06307	HPA069515	1:200
CD200	CD200 molecule	P41217	HPA031149	1:25
CD99L2	CD99 molecule like 2	Q8TCZ2	HPA061400	1:200
CDH8	Cadherin 8	P55286	HPA014908	1:200
CGREF1	Cell growth regulator with EF-hand domain 1	Q99674	HPA008241	1:200
CHIT1	Chitinase 1	Q13231	HPA010575	1:25
CHL1	Cell adhesion molecule L1 like	O00533	HPA003345	1:200
CLSTN1	Calsyntenin 1	O94985	HPA012412	1:200
CLSTN3	Calsyntenin 3	Q9BQT9	HPA070830	1:200
CNDP1	Carnosine dipeptidase 1	Q96KN2	HPA016933	1:200
CNTN1	Contactin 1	Q12860	HPA070467	1:25
CPNE6	Copine 6	O95741	HPA031636	1:200
DACT3	Dishevelled binding antagonist of beta catenin 3	Q96B18	HPA043053	1:200
DDAH1	Dimethylarginine dimethylaminohydrolase 1	O94760	HPA006308	1:25
DMTN	Dematin actin binding protein	Q08495	HPA024290	1:200
DOCK10	Dedicator of cytokinesis 10	Q96BY6	HPA058106	1:200
GAP43	Growth associated protein 43	P17677	HPA013603	1:200
GFAP	Glial fibrillary acidic protein	P14136	16825-1-AP	1:25
HEPN1	Hepatocellular carcinoma, down-regulated 1	Q6WQI6	HPA063054	1:25
IGLON5	IgLON family member 5	A6NGN9	HPA041994	1:25
ITIH1	Inter-alpha-trypsin inhibitor heavy chain 1	P19827	HPA042049	1:200
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	Q9H008	HPA009269	1:25
LINGO1	Leucine rich repeat and Ig domain containing 1	Q96FE5	HPA074653	1:200
LY6H	Lymphocyte antigen 6 family member H	O94772	HPA077218	1:25
MACIR	Macrophage immunometabolism regulator	Q96GV9	HPA043434	1:25
MAP1B	Microtubule associated protein 1B	P46821	HPA022275	1:25
MAP7	Microtubule associated protein 7	Q14244	HPA029712	1:25
MARCKSL1	MARCKS like 1	P49006	HPA030528	1:25
MBP	Myelin basic protein	P02686	HPA049222	1:25
MOG	Myelin oligodendrocyte glycoprotein	Q16653	HPA021873	1:200
NCAN	Neurocan	O14594	HPA058000	1:200
NECAB2	N-terminal EF-hand calcium binding protein 2	Q7Z6G3	HPA013998	1:25
NEFM	Neurofilament medium	P07197	HPA022845	1:25
NPM2	Nucleophosmin/nucleoplasmin 2	Q86SE8	HPA041070	1:25
NPTX2	Neuronal pentraxin 2	P47972	HPA058320	1:200
NPTXR	Neuronal pentraxin receptor	O95502	HPA001079	1:25
NRCAM	Neuronal cell adhesion molecule	Q92823	HPA061433	1:25

HGNC ID	Protein name	Uniprot ID	Antibody	Sample dilution
NRGN	Neurogranin	Q92686	HPA038171	1:25
NRXN3	Neurexin 3	Q9HDB5, Q9Y4C0	HPA002727	1:200
NT5E	5'-nucleotidase ecto	P21589	HPA048043	1:25
OMG	Oligodendrocyte myelin glycoprotein	P23515	HPA008206	1:200
PAM	Peptidylglycine alpha-amidating monooxygenase	P19021	HPA042260	1:200
PCSK1N	Proprotein convertase subtilisin/kexin type 1 inhibitor	Q9UHG2	HPA064734	1:200
PDYN	Prodynorphin	P01213	HPA053342	1:200
PEBP1	Phosphatidylethanolamine binding protein 1	P30086	HPA063904	1:200
PTPRN2	Protein tyrosine phosphatase receptor type N2	Q92932	HPA007255	1:200
QDPR	Quinoid dihydropteridine reductase	P09417	HPA058951	1:25
RPH3A	Rabphilin 3A	Q9Y2J0	HPA002475	1:25
RTN4R	Reticulon 4 receptor	Q9BZR6	HPA063584	1:200
SCG3	Secretogranin III	Q8WXD2	HPA053715	1:200
SERPINA3	Serpin family A member 3	P01011	HPA000893	1:200
SNCB	Synuclein beta	Q16143	HPA035876	1:200
SPECC1	Sperm antigen with calponin homology and coiled-coil domains 1	Q5M775	HPA021421	1:25
STMN3	Stathmin 3	Q9NZ72	HPA012947	1:25
SYT11	Synaptotagmin 11	Q9BT88	HPA064091	1:25
TARDBP	TAR DNA binding protein	Q13148	10782-2-AP	1:25
TESPA1	Thymocyte expressed, positive selection associated 1	A2RU30	HPA058823	1:200
TFRC	Transferrin receptor	P02786	HPA028598	1:200
TMEM132D	Transmembrane protein 132D	Q14C87	HPA010739	1:25
TXNDC17	Thioredoxin domain containing 17	Q9BRA2	HPA022931	1:25
VGF	VGF nerve growth factor inducible	O15240	HPA058371	1:200

Supplementary table 2: *p*-values from Dunn's test comparing median CSF levels across diagnostic groups

comparison	p-value	adjusted p-value	significant
AD - ALS	0.734	0.856	no
AD - CBS	0.051	0.133	no
AD - Healthy control	0.046	0.160	no
AD - PPA	0.286	0.462	no
AD - PSP	0.003	0.059	no
AD - bvFTD	0.003	0.030	yes
ALS - CBS	0.143	0.334	no
ALS - Healthy control	0.150	0.316	no
ALS - PPA	0.658	0.813	no
ALS - PSP	0.023	0.098	no
ALS - bvFTD	0.049	0.148	no
CBS - Healthy control	0.892	0.937	no
CBS - PPA	0.159	0.304	no
CBS - PSP	0.557	0.731	no
Healthy control - PPA	0.160	0.279	no
Healthy control - PSP	0.429	0.601	no
PPA - PSP	0.012	0.086	no
bvFTD - CBS	0.983	0.983	no
bvFTD - Healthy control	0.872	0.964	no
bvFTD - PPA	0.013	0.067	no
bvFTD - PSP	0.401	0.602	no

Supplementary table 3 and supplementary table 4:

Supplementary table 3: Top five elevated and decreased proteins per disease with the best performance in separating the affected individuals from healthy controls when adjusted for median CSF protein levels.

		AD protein	AD AUC	ALS protein	ALS AUC	CBS protein	CBS AUC	PPA protein	PPA AUC	PSP protein	PSP AUC	bvFTD protein	bvFTD AUC
elevated in disease		GAP43	0.92	NEFM	0.92	NEFM	0.85	NEFM	0.88	MARCKSL1	0.84	GAP43	0.8
		AMPH	0.89	MAP1B	0.88	GAP43	0.83	GAP43	0.86	NEFM	0.84	MAP1B	0.79
		ARPP21	0.85	SNCB	0.85	MARCKSL1	0.82	AMPH	0.82	CALB2	0.81	AMPH	0.78
		MAP1B	0.82	AMPH	0.83	LHPP	0.81	MAP1B	0.79	GAP43	0.76	MARCKSL1	0.77
		NRGN	0.82	MARCKSL1	0.82	AMPH	0.81	MARCKSL1	0.79	AMPH	0.75	QDPR	0.76
decreased in disease		PCSK1N	0.9	LINGO1	0.84	LINGO1	0.89	PCSK1N	0.87	LINGO1	0.88	LINGO1	0.86
		LINGO1	0.89	PCSK1N	0.83	PCSK1N	0.87	LINGO1	0.86	PCSK1N	0.87	PCSK1N	0.85
		PDYN	0.88	PDYN	0.82	PTPRN2	0.83	PDYN	0.84	PDYN	0.83	PTPRN2	0.82
		PTPRN2	0.85	NPTX2	0.81	PDYN	0.81	PTPRN2	0.81	CCK	0.81	PDYN	0.81
		CCK	0.82	PTPRN2	0.8	CCK	0.81	CCK	0.8	PTPRN2	0.8	CCK	0.81

Supplementary table 4: Number of individuals per AT group and diagnosis.

Diagnosis	AT status A+T+	A+T-	A-T+	A-T-
AD	55	3	3	8
ALS	4	2	3	26
CBS	5	4	4	13
PPA	51	8	11	96
PSP	6	3	1	29
bvFTD	21	10	12	86
total	142	30	34	258