

A peripheral proteomic signature of Alzheimer’s disease is identified in the plasma extracellular vesicles of mild cognitive impairment patients from a memory clinic: the BIOPEXAL study

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Figures

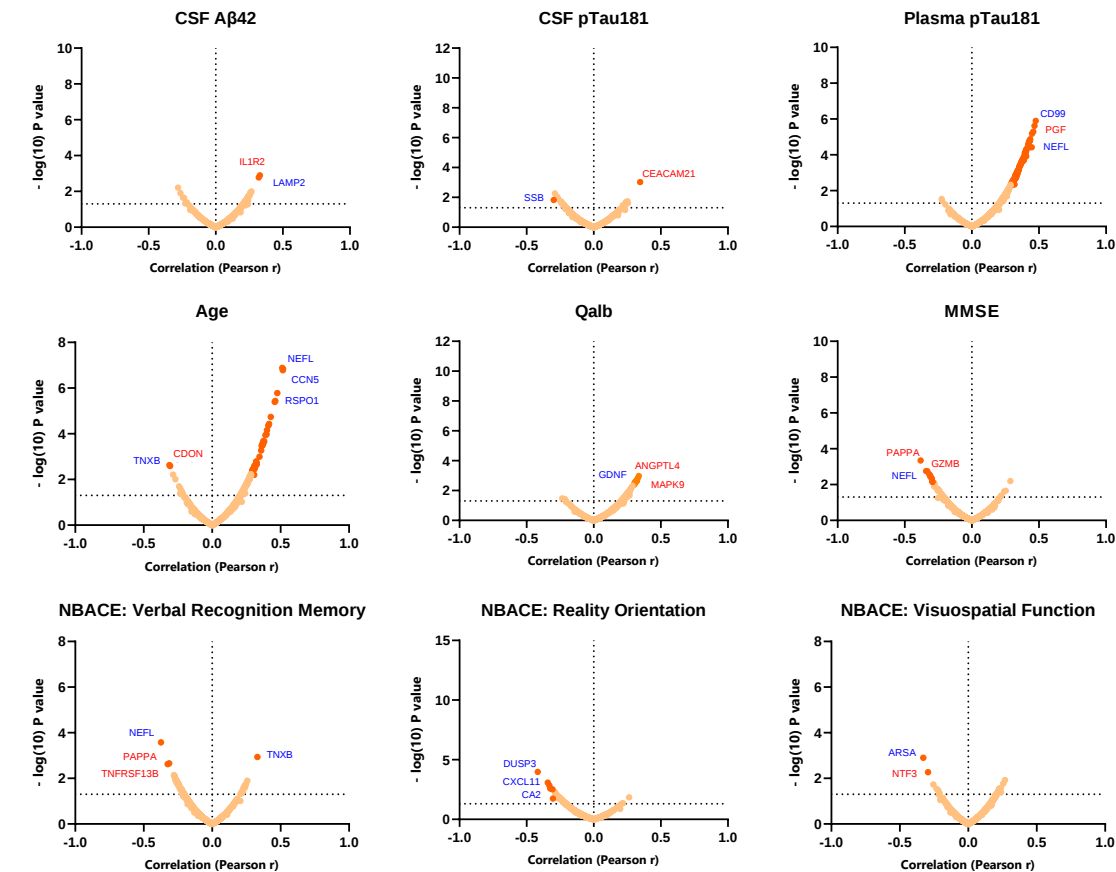


Figure S1. Volcano plots of the associations between serum protein levels and main AD clinical variables. Graph is expressed by the magnitude of correlation ($-\log(10) p \text{ Value}$) vs fold-change, expressed by Pearson r , of the correlation. Dark dots refer to proteins with Pearson $r > 0.30$. Horizontal grid line represents the significant $p \text{ Value}$ threshold ($p < 0.05$). Proteins labelled in blue belongs to the neurology panel. Proteins labelled in red belongs to the inflammation panel.

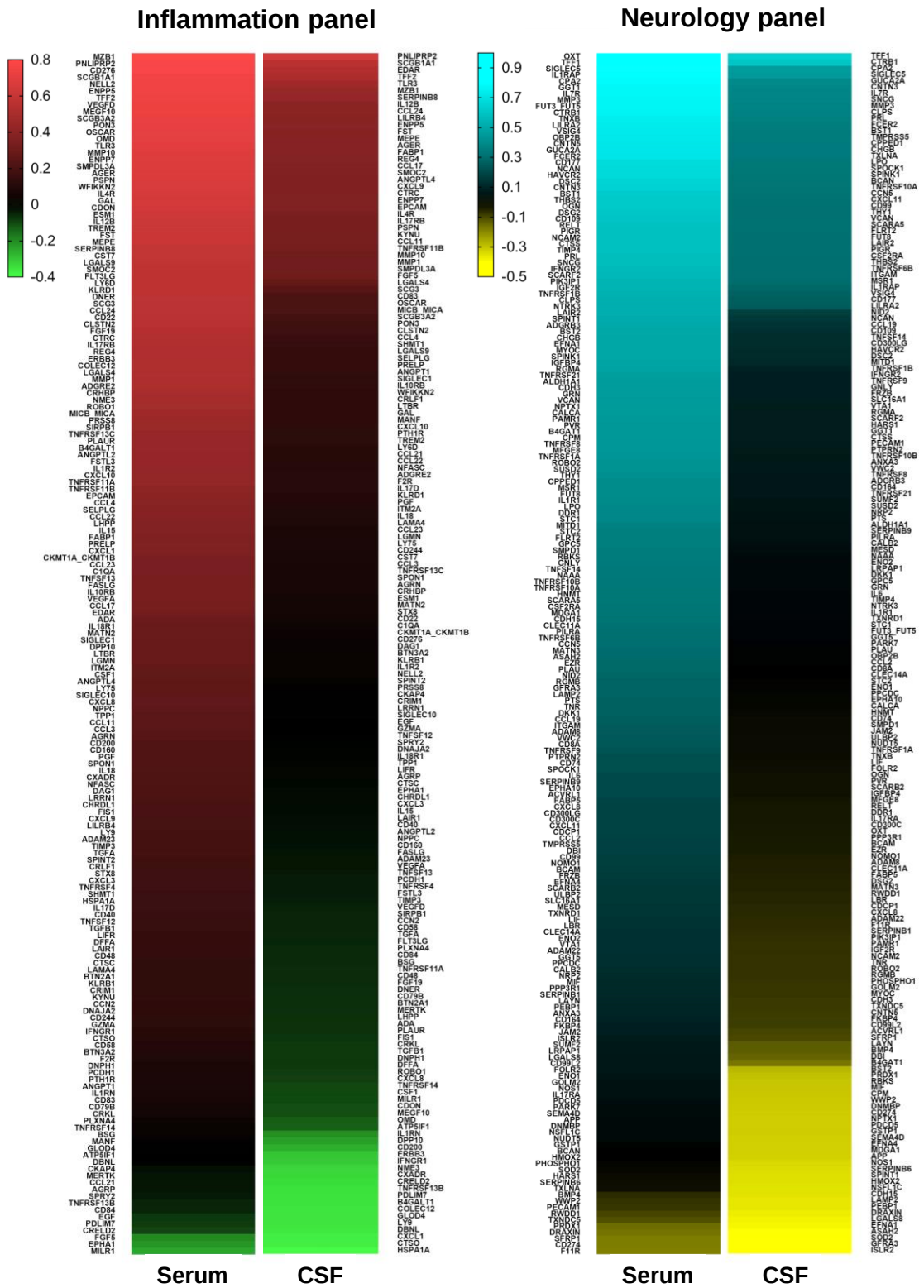


Figure S2. Heatmaps of the correlations between pEVs proteins and their CSF and serum homologues in both Inflammation I and Neurology I Olink® Explore panels.

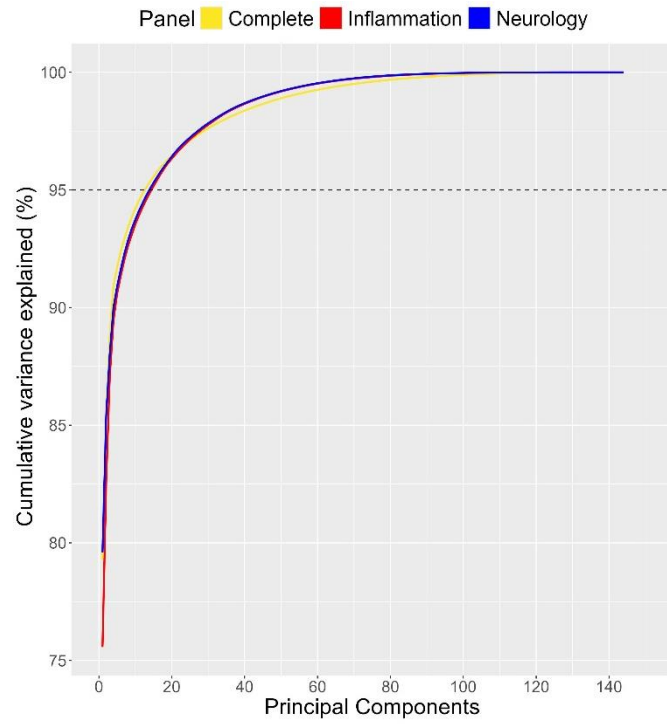


Figure S3. The cumulative variance explained (%) of the PCA.

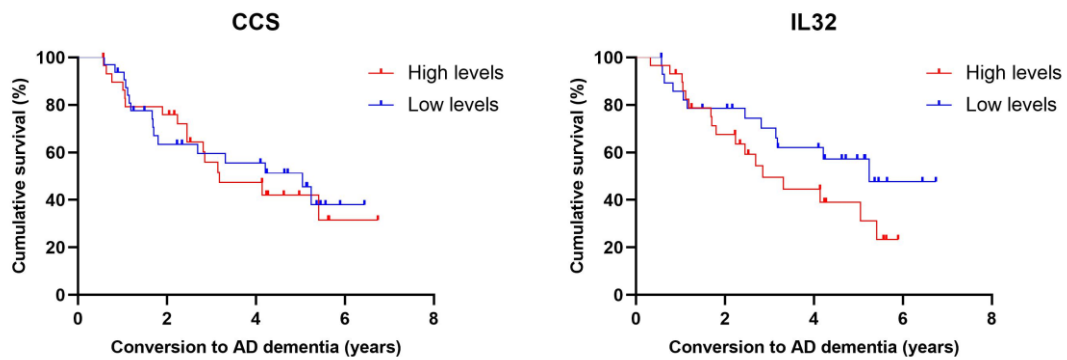


Figure S4. Survival curves from *Kaplan-Meier* analysis of serum proteins that significantly predicted the MCI conversion to AD dementia in the pEVs compartment. "Accumulated survival" refers to the time until conversion to AD dementia.

Tables

Table S1. One-way ANOVA - Tukey's multiple comparisons test of top six main proteins in both neurology and inflammation Olink® Explore panels.

One way ANOVA test - Tukey's multiple comparisons						
Protein	Study groups' comparison	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adj p Value
Neurology pathway						
APP	MCI A+T+ vs. MCI A-T-	0.6877	0.1378 to 1.238	Yes	**	0.00999162
	AD dementia vs. MCI A-T-	1.832	1.272 to 2.391	Yes	****	5.493E-12
	AD dementia vs. MCI A+T+	1.144	0.5819 to 1.706	Yes	****	1.1149E-05
CHMP1A	MCI A+T+ vs. MCI A-T-	0.5448	0.1962 to 0.8933	Yes	***	0.0009081
	AD dementia vs. MCI A-T-	1.54	1.190 to 1.891	Yes	****	<1.0000E-15
	AD dementia vs. MCI A+T+	0.9954	0.6554 to 1.336	Yes	****	5.4406E-10
IGF2R	MCI A+T+ vs. MCI A-T-	0.22	0.02892 to 0.4096	Yes	*	0.01951462
	AD dementia vs. MCI A-T-	0.41	0.2188 to 0.6102	Yes	****	4.6619E-06
	AD dementia vs. MCI A+T+	0.20	-0.001381 to 0.3919	No	ns	0.05207556
MAX	MCI A+T+ vs. MCI A-T-	0.4378	0.09045 to 0.7852	Yes	**	0.00936903
	AD dementia vs. MCI A-T-	1.213	0.8583 to 1.567	Yes	****	1.189E-12
	AD dementia vs. MCI A+T+	0.775	0.4390 to 1.111	Yes	****	7.0162E-07
MMP8	MCI A+T+ vs. MCI A-T-	0.69	0.2514 to 1.123	Yes	***	0.00078802
	AD dementia vs. MCI A-T-	0.76	0.3119 to 1.212	Yes	***	0.00028833
	AD dementia vs. MCI A+T+	0.08	-0.3752 to 0.5253	No	ns	0.9177435
NDRG1	MCI A+T+ vs. MCI A-T-	0.29	0.04081 to 0.5460	Yes	*	0.01833832
	AD dementia vs. MCI A-T-	0.52	0.2587 to 0.7757	Yes	****	1.5789E-05
	AD dementia vs. MCI A+T+	0.22	-0.03601 to 0.4835	No	ns	0.10634463
Inflammation pathway						
ESM1	MCI A+T+ vs. MCI A-T-	0.27	0.04091 to 0.4954	Yes	*	0.01620526
	AD dementia vs. MCI A-T-	0.44	0.2017 to 0.6804	Yes	****	7.2977E-05
	AD dementia vs. MCI A+T+	0.17	-0.06644 to 0.4123	No	ns	0.2044727
HLADRA	MCI A+T+ vs. MCI Aβ(-)	-0.18	-0.4193 to 0.06347	No	ns	0.1915
	AD dementia vs. MCI A-T-	-0.41	-0.6668 to -0.1432	Yes	**	0.001
	AD dementia vs. MCI A+T+	-0.23	-0.4889 to 0.03469	No	ns	0.103
IL1R2	MCI A+T+ vs. MCI A-T-	-0.21	-0.3919 to -0.01981	Yes	*	0.0262
	AD dementia vs. MCI A-T-	-0.36	-0.5485 to -0.1616	Yes	****	7.7689E-05
	AD dementia vs. MCI A+T+	-0.15	-0.3417 to 0.04333	No	ns	0.1618
IL32	MCI A+T+ vs. MCI A-T-	0.29	0.06666 to 0.5049	Yes	**	0.0068
	AD dementia vs. MCI A-T-	0.30	0.07592 to 0.5295	Yes	**	0.0054
	AD dementia vs. MCI A+T+	0.02	-0.2099 to 0.2438	No	ns	0.9829
PRSS8	MCI A+T+ vs. MCI A-T-	-0.39	-0.7741 to -0.002661	Yes	*	0.048
	AD dementia vs. MCI A-T-	-0.69	-1.090 to -0.2917	Yes	***	0.0002
	AD dementia vs. MCI A+T+	-0.30	-0.6996 to 0.09475	No	ns	0.172

SMOC2	MCI A+T+ vs. MCI A-T-	0.16	0.03559 to 0.2840	Yes	**	0.0077
	AD dementia vs. MCI A-T-	0.24	0.1115 to 0.3686	Yes	****	5.8314E-05
	AD dementia vs. MCI A+T+	0.08	-0.04830 to 0.2088	No	ns	0.304

Table S2. Top 5 correlated pEVs proteins with main AD clinical features. Uniprot database was used for the identification of related molecular pathways and main protein functions. Proteins labelled in blue belongs to the neurology panel. Proteins labelled in red belongs to the inflammation panel.

Main AD clinical variables	Protein	Pearson r	95% CI	P (two-tailed)	Main Functions and Involved Pathways
CSF A β 42	PIK3AP1	-0,4097	-0,5216 to -0,2839	4,3606E-09	Akt signaling pathway
	CHMP1A	-0,3946	-0,5142 to -0,2599	9,26599E-08	Multivesicular bodies (MVBs) formation pathway
	ANXA11*	-0,3946	-0,5084 to -0,2672	1,76723E-08	Cytokinesis and vesicle-mediated transport
	TRAF2*	-0,3913	-0,5061 to -0,2629	2,81723E-08	TNF receptor-associated factor
	NFATC1*	-0,3888	-0,5060 to -0,2576	6,34062E-08	Inducible expression of cytokine genes in T-cells (IL-2 / IL-4)
CSF pTau181	ATP5PO	0,4759	0,3314 to 0,5986	9,19647E-09	Mitochondrial membrane ATP synthase. Nucleobase-containing small molecule metabolic process
	NFATC1*	0,4654	0,3428 to 0,5724	4,0646E-11	Inducible expression of cytokine genes in T-cells (IL-2 / IL-4)
	CHMP1A*	0,4591	0,3319 to 0,5699	2,6994E-10	Multivesicular bodies (MVBs) formation, Cytoskeleton and membrane organization.
	BCR*	0,4576	0,3242 to 0,5731	1,69039E-09	Signaling and molecular function regulator activity
	TRAF2*	0,4547	0,3333 to 0,5613	5,5785E-11	TNF receptor-associated factor
plasma pTau181	RABGAP1L	0,4184	0,2863 to 0,5349	1,24036E-08	Vesicle-mediated transport and cell differentiation
	TBC1D17	0,4081	0,2110 to 0,5733	0,000128431	Autophagy regulation and vesicle-mediated transport
	CXCL8	0,4024	0,2747 to 0,5161	1,14077E-08	Chemotaxis, inflammatory response, immune system process, vesicle-mediated transport, signaling and cell motility.
	DUSP3	0,3993	0,2527 to 0,5279	6,52858E-07	Cytoskeletal protein binding, immune system process, signaling and cell adhesion, and cell motility.
	CXCL11*	0,398	0,2710 to 0,5114	1,29235E-08	Important role in CNS diseases involving T-cell recruitment. Inflammatory response, membrane transport, signaling and cell motility.
Qalb	AKT1S1	0,4747	0,3551 to 0,5791	7,609E-12	Involved in nerve growth factor-mediated neuroprotection. Programmed cell death and signaling.
	WWP2	0,4746	0,3563 to 0,5779	4,578E-12	Transcription regulator activity, protein catabolic process, and transmembrane transport.
	BSG	0,4549	0,3331 to 0,5617	6,1408E-11	Potent stimulator of IL6 secretion, VEGFA-mediated activation and downstream signaling, angiogenesis, immune system process, signaling, anatomical structure development, and cell motility.
	IKBKG	0,4544	0,3336 to 0,5605	4,5548E-11	NF-kappa-B essential modulator
	ANXA11*	0,4523	0,3312 to 0,5586	5,7379E-11	Cytokinesis and vesicle-mediated transport
Age	CXCL10	0,3668	0,2368 to 0,4839	1,9366E-07	Important role in neurons for activating microglia. Chemotaxis, differentiation, and activation of peripheral immune cells, regulation of cell growth, apoptosis and modulation of angiostatic effects.
	IGFBP4	0,3509	0,2192 to 0,4701	7,3836E-07	Involved in insulin pathway and cell signaling.
	VSIG4	0,3341	0,2002 to 0,4558	2,9641E-06	Phagocytic receptor, cell adhesion, strong negative regulator of T-cell proliferation and IL2 production.
	CXCL11*	0,3195	0,1856 to 0,4417	7,0295E-06	Important role in CNS diseases involving T-cell recruitment. Inflammatory response, membrane transport, signaling and cell motility.
	IL17C	0,3096	0,1592 to 0,4459	9,3595E-05	Stimulates pro-inflammatory molecules by signaling through the NF-kappa-B and MAPK pathways.
MMSE	TARBP2*	-0,4178	-0,5422 to -0,2756	1,1445E-07	RNA binding, protein catalytic activity, immune system process, and signaling.
	NFATC1*	-0,415	-0,5306 to -0,2841	1,1328E-08	Involved in the inducible expression of cytokine genes in T-cells (especially IL-2 and IL-4). Signaling, cell differentiation, anatomical structure development.
	PTEN	-0,3863	-0,5279 to -0,2240	1,1063E-05	Protein and lipid phosphatase. Modulator of the AKT-mTOR signaling pathway (controlling neurogenesis, dendritic development and synapse formation)
	CHMP1A*	-0,3854	-0,5079 to -0,2476	2,9431E-07	Promotes cell polarization and directed movement.
	DNMBP	-0,3828	-0,5001 to -0,2518	8,8581E-08	Multivesicular bodies (MVBs) formation, Cytoskeleton and membrane organization.
					Critical role in several intracellular processes associated with the microtubule cytoskeleton and

					membrane trafficking between the cell surface and the Golgi.
NBACE Reality Orientation	CCN2	-0,4998	-0,6000 to -0,3842	2,83E-13	Major connective tissue mitotractant secreted by vascular endothelial cells (BBB). Cytoskeleton organization, signaling, cell differentiation.
	NUB1	-0,4962	-0,5987 to -0,3777	1,222E-12	Involved in immune system and protein catalysis processes.
	ARHGEF12	-0,4888	-0,5938 to -0,3674	5,876E-12	GTPase-activating protein, signaling and molecular function regulator activity.
	WASF3	-0,4877	-0,6092 to -0,3438	4,5928E-09	Involved in the regulation of cell morphology and cytoskeletal organization. Transmission of signals from tyrosine kinase receptors and small GTPases to the actin cytoskeleton.
	BCR*	-0,4874	-0,5990 to -0,3572	1,2654E-10	Signaling and molecular function regulator activity.
NBACE Visuospatial Function	FKBP1B*	-0,3951	-0,5601 to -0,2000	0,00016661	Isomerase and cell transport activity. Immune system process and protein folding.
	TRIM5	-0,3912	-0,5093 to -0,2587	7,2756E-08	Activation of the MAP3K7/TAK1 complex, induction and expression of NF-kappa-B and MAPK-responsive inflammatory genes, induction an innate immune response.
	TARBP2*	-0,3908	-0,5186 to -0,2460	7,6166E-07	RNA binding, protein catalytic activity, immune system process, and signaling.
	PRKAB1	-0,3869	-0,5164 to -0,2400	1,2966E-06	Involved in AMPK pathway. Inhibits protein, carbohydrate and lipid biosynthesis, as well as cell growth and proliferation. Regulator of cellular polarity by remodeling the actin cytoskeleton.
	TBC1D5	-0,3847	-0,5243 to -0,2250	8,6703E-06	GTPase-activating protein. Transport from endosomal membrane to the cytosol and to the trans-Golgi network. Catalytic activity and autophagy. Involved in reprogramming vesicle trafficking.
NBACE Verbal Recognition Memory	FKBP1B*	-0,4714	-0,6212 to -0,2883	4,6267E-06	Isomerase and cell transport activity. Immune system process and protein folding.
	TARBP2*	-0,3983	-0,5247 to -0,2548	4,0816E-07	RNA binding, protein catalytic activity, immune system process, and signaling.
	IMPA1	-0,3943	-0,5258 to -0,2443	1,4362E-06	Phosphatase involved in the dephosphorylation of myoinositol. Involved in maintaining normal brain function.
	NFATC1*	-0,3911	-0,5080 to -0,2601	5,2212E-08	Involved in the inducible expression of cytokine genes in T-cells (especially IL-2 and IL-4). Signaling, cell differentiation, anatomical structure development.
	MAP2K6	-0,3858	-0,5065 to -0,2502	1,8785E-07	Essential component of the MAP kinase signal transduction pathway. Important role in the regulation of cellular responses to cytokines.

*Proteins significantly associated with two or more AD clinical features.

Table S3. Obtained results from Kaplan Meier analysis of serum proteins ability to predict the conversion to AD dementia. Table shows the proteins that exhibited significance on the analysis.

Comparison of Survival Curves	CCS	IL32
Log-rank (Mantel-Cox) test		
Chi square	0,09595	1,908
P value	0,7567	0,1672
P value summary	ns	ns
Are the survival curves sig different?	No	No
Median survival (years)		
High levels	3,176	2,845
Low levels	5,046	5,243
Hazard Ratio (Mantel-Haenszel)		
Ratio	1,117	1,679
95% CI of ratio	0,5557 to 2,244	0,8049 to 3,502